# Glutathione-mediated detoxification systems in plants

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Recent work has highlighted the presence of diverse glutathione-dependent enzymes in plants with potential roles in the detoxification of both xenobiotic and endogenous compounds. In particular, studies on glutathione transferases are further characterising their role in xenobiotic metabolism, and also raising intriguing possible roles in endogenous metabolism. The solution of their three-dimensional structures together with studies on their molecular diversity and substrate specificity is providing new insights into the function and classification of these enigmatic enzymes.

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**Current Opinion in Plant Biology** 1998, 1:258–266

http://biomednet.com/elecref/1369526600100258



**Abbreviations**

| | |
|---|---|
| **ABC** | ATP-binding cassette |
| **G-site** | glutathione-binding site |
| **GSH** | reduced glutathione |
| **GSSG** | oxidised glutathione |
| **GST** | glutathione transferase |
| **GPOX** | glutathione peroxidase |
| **PHGPX** | phospholipid hydroperoxide glutathione peroxidase |

## Introduction

The tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) is the most abundant form of organic sulphur in plants apart from that incorporated into proteins. Under normal conditions, glutathione is predominantly present in its reduced form (GSH), with only a small proportion present in its fully oxidised state (GSSG). Unlike animals, plants also synthesise analogous tripeptides such as γ-glutamyl-cysteinyl-β-alanine (homoglutathione) which is found in several legume species and γ-glutamyl-cysteinyl-serine and γ-glutamyl-cysteinyl-glutamate which have been reported in cereals [1•]. In addition to functioning as a translocatable store of organic sulphur [1•], recent studies suggest that all of these forms of glutathione have multifunctional roles in cellular metabolism, notably as products of primary assimilation [2], as reductants and radical scavenging agents [3], and as reactive nucleophiles used in the detoxification of electrophilic toxins. It also appears that glutathione can function as an intracellular signalling agent, responsive to changes in the extracellular environment [4]. In this short review we will concentrate on the recent evidence suggesting that glutathione and several of its dependent enzymes are central to detoxifying reactive low molecular weight organic compounds of xenobiotic or endogenous origins. In some cases, these protective functions appear to be highly conserved with those found in animals, but in others, plants may have evolved unique and, as yet, poorly defined roles for these detoxification systems. This review will discuss in detail recent studies on glutathione transferases, the best characterised group of glutathione-dependent enzymes in plants, together with recently elucidated details of the less well known groups of glutathione-dependent enzymes: formaldehyde dehydrogenases, glyoxalases and glutathione peroxidases. Although all of these enzymes have been previously described in animals, their functions in plant metabolism are far from clear. The possible roles of these enzymes in xenobiotic and endogenous metabolism, and the evidence for these activities, will be discussed.

## Role of glutathione in formaldehyde detoxification

Formaldehyde is a reactive molecule, commonly encountered as a product of both endogenous metabolism and as an environmental pollutant. In animals formaldehyde is rapidly detoxified by the glutathione-dependent formaldehyde dehydrogenase system, which is composed of two enzymes. In the first reaction, glutathione spontaneously reacts with formaldehyde to form *S*-hydroxymethylglutathione. The enzyme formaldehyde dehydrogenase then catalyses the formation of *S*-formylglutathione, i.e. overall:

$$GSH + HCHO + NAD^+ \rightarrow GS\text{-}CHO + NADH + H^+.$$

In the second reaction the thiolester is hydrolysed by *S*-formylglutathione hydrolase to release formic acid and glutathione:

$$GS\text{-}CHO + H_2O \rightarrow GSH + HCOOH.$$

The first enzyme in this pathway, formaldehyde dehydrogenase, has recently been characterised at the molecular level in maize [5] and peas [6] and shown to be a 41 kDa enzyme. This enzyme is closely related to the class III alcohol dehydrogenases, which are a group of zinc-containing dimeric alcohol dehydrogenases widely distributed in the animal kingdom, and contains highly conserved catalytic domains. Although this detoxification pathway has not yet been demonstrated to function *in vivo* in plants, maize mRNA encoding the enzyme is actively expressed in dividing suspension-cultured cells [5]. It seems very likely that in view of the conservation of sequence of formaldehyde dehydrogenase between plants and animals that the system plays a similarly important role in formaldehyde metabolism in both systems.

## The glyoxalase pathway

In addition to formaldehyde, actively respiring plant cells generate other reactive aldehydes, notably methylglyoxal,

which is formed from the metabolism of triose phosphates, acetone and threonine. Methylglyoxal is highly toxic, reacting with both DNA and protein and is detoxified by a two-step enzyme catalysed process, which shows some similarities to the formaldehyde dehydrogenase system. Methylglyoxal first spontaneously reacts with glutathione to form a hemithioacetal derivative which is then converted to *S*-lactoylglutathione by the enzyme glyoxalase I:

$CH_3COCHO + GSH \rightarrow CH_3COCH(OH)\text{-}SG \rightarrow CH_3CH(OH)CO\text{-}SG.$

The *S*-lactoylglutathione is then hydrolysed by glyoxalase II to release the non-toxic lactic acid and glutathione:

$CH_3CH(OH)CO\text{-}SG \rightarrow CH_3CH(OH)COOH + GSH.$

The two enzymes comprising this shunt pathway of metabolism have been identified in both plants and animals for some time, being particularly associated with rapid cell division, though the exact function of the glyoxalase pathway remains unknown. Recently both glyoxalases from plants have been subject to molecular characterisation. Glyoxalase I has been cloned from tomato and shown to encode a functionally active 21 kDa polypeptide with extensive sequence identity at the amino acid level to glyoxalase I from bacteria and mammals [7]. Antiserum raised to glyoxalase I showed that the enzyme was expressed in all cell types, particularly in the phloem and sieve elements. Transcripts encoding glyoxalase I accumulated in tomato in response to treatment with sodium chloride, mannitol and abscisic acid, suggesting that glyoxalase I was required during water stress as well as during cell division. An additional nucleotide sequence described as encoding a glyoxalase I from soybean is also registered on the GenBank database (accession number X68819). The identity of this cDNA, however, appears to have been mis-assigned and we have recently demonstrated that it encodes a glutathione transferase subunit [8].

Glyoxalase II has also recently been independently cloned from *Arabidopsis thaliana* by two research groups [9,10]. As with glyoxalase I, the glyoxalase II cDNAs showed extensive sequence identity to the respective mammalian enzyme. This similarity was further demonstrated by showing that the recombinant 29 kDa glyoxalase II from *A. thaliana* hydrolysed a similar range of thiol esters to the enzyme from humans [9]. Glyoxalase II in *A. thaliana* was shown to be encoded by two gene families, one of which encoded a glyoxalase II that was expressed in the cytosol while the other encoded glyoxalase II with a transit peptide sequence for deposition in mitochondria [10]. Interestingly, the two forms of glyoxalase II were separately regulated, with the mitochondrial form being most highly expressed in the roots and the cytosolic form preferentially expressed in the flower buds.

Collectively these recent studies give further evidence of the importance of this neglected shunt pathway of primary metabolism. The observation of two compartmentalised forms of glyoxalase II was particularly significant, as the glyoxalase pathway is known to operate in both the cytosol and mitochondria in animal cells. It will now be of interest to determine whether or not glyoxalase I is encoded by multiple genes, and if any of these genes also contain mitochondrial targeting sequences.

## Glutathione transferases

Glutathione transferases, also termed glutathione *S*-transferases (GSTs), are ubiquitous in aerobic organisms and catalyse the conjugation of electrophilic and frequently hydrophobic toxic compounds with glutathione to form non-toxic peptide derivatives. The toxic electrophile may be of xenobiotic or natural origin and in plants GSTs have been well studied with respect to their ability to detoxify herbicides, with the most commonly observed conjugations involving substitution reactions, such as:

$R\text{-}X + GSH \rightarrow R\text{-}SG + HX.$

GSTs can also catalyse addition reactions with glutathione, though these have been less frequently observed with xenobiotic substrates in plants.

Recent studies have unambiguously confirmed the importance of glutathione conjugation reactions in herbicide selectivity by showing that both cereal [11] and broadleaf [12] crops containing high GST activities toward herbicides are tolerant, whereas competing weeds with lower GST activities are susceptible. The importance of GSTs in herbicide tolerance has been further confirmed by demonstrating that transgenic tobacco plants expressing a herbicide-detoxifying GST from maize are more tolerant of chloroacetanilide and thiocarbamate herbicides than untransformed controls [13].

All plant GSTs have native relative molecular masses of around 50 kDa and are composed of two similarly sized (~25kDa) subunits. Each subunit contains a kinetically independent active site with distinct binding domains for glutathione and the co-substrate [14••]. These subunits may be identical, giving rise to homodimers, or distinct but related, resulting in heterodimers, with each distinct subunit encoded by a different gene. Since plants contain complex multigene families of GSTs, the various subunits may be able to dimerise in many permutations, producing multiple homo- and hetero-dimeric GST isoenzymes in the plant. The GST isoenzymes involved in xenobiotic metabolism are subject to discreet regulation, showing distinct, but overlapping, substrate specificities [15,16].

Although GSTs were originally studied in plants because of their involvement in herbicide detoxification there has recently been intense interest in the roles they play in endogenous metabolism of plants [14••]. This interest

has been stimulated by the observations that GSTs are unusually abundant and are subject to complex spatial and temporal regulation in response to plant development and numerous stress treatments [14••].

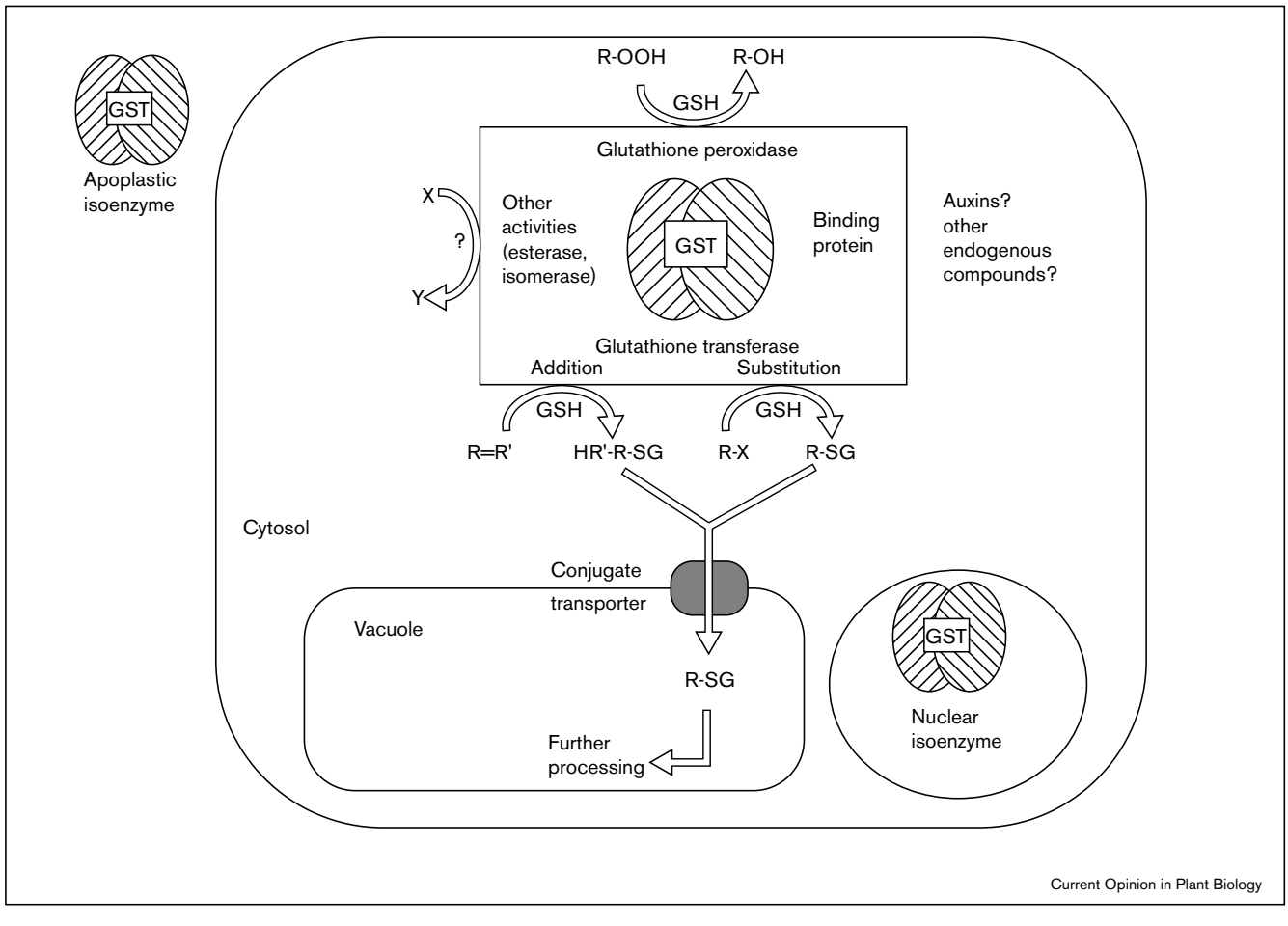
The cellular and subcellular localisation of GSTs has not been studied in great detail, though they are most commonly believed to be cytoplasmic enzymes [17]. There are reports, however, of specific isoenzymes being expressed in the apoplast [18], and in tobacco the auxin-inducible Nt ParA GST has been identified in the nucleus ([19], Figure 1). Assuming a cytosolic location for most GSTs, it is significant that a complex family of ATP-binding cassette (ABC) transporter proteins capable of selectively transporting the glutathione conjugated products formed by GSTs have recently been identified in the tonoplast membrane [20•,21]. As glutathione conjugates are often potent competitive inhibitors of GSTs these ABC-transporters fulfil an important role in removing the potentially inhibitory reaction products and sequestering them in the vacuole (Figure 1). Once in the vacuole, these conjugates then undergo further processing to a complex range of *S*-linked peptide derivatives [22].

The functions and regulation of plant GSTs have been comprehensively reviewed relatively recently [14••]. In this review, we will concentrate on more recent findings, including plant GST classification, crystal structure and enzyme activity.

### Classification of plant GSTs

Within the existing nomenclature system of alpha, mu, pi, theta and zeta class GSTs established in animals, it has been considered that all plant GSTs were members of the theta class, an evolutionarily ancient and heterogeneous class of GSTs comprising most non-mammalian GSTs

**Figure 1**

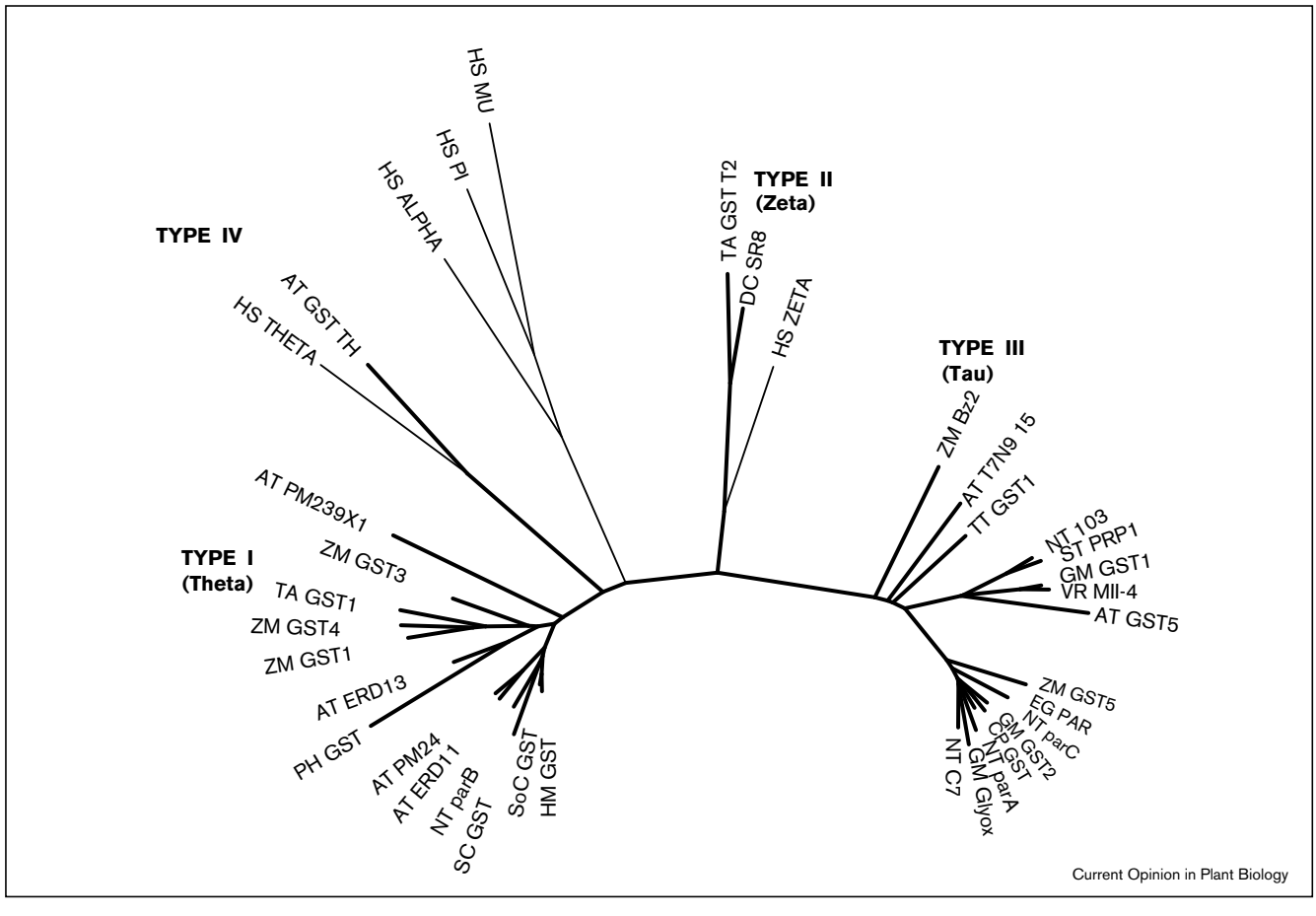



Overview of the roles of plant GSTs in xenobiotic detoxification and endogenous metabolism. Most plant GSTs are assumed to be cytosolic, although there is evidence for apoplastic and nuclear isoenzymes. The primary transferase activity of GSTs results in glutathione conjugation of the substrate, usually in a substitution reaction but occasionally as an addition reaction; conjugated molecules (R-SG) are then transported into the vacuole for further processing. There is evidence for alternative activities of GSTs; these include glutathione peroxidase, esterase, isomerase and binding activities, which may play additional roles in endogenous metabolism.

together with mammalian homologues [14••]. It has now been recognised, however, that this classification system is too simplistic to categorise the rapidly increasing number and diversity of plant GST nucleotide sequences being deposited in the databases and there is now evidence for at least four distinct GST classes in plants. A phylogenetic relationship for plant GSTs, proposed by the authors, and derived from deduced polypeptide sequences, also showing their relationship to the mammalian GSTs, is shown in Figure 2. In the first attempt to classify plant GSTs, Droog *et al* proposed three types, on the basis of polypeptide sequence similarity and exon structure [23].

Type I GSTs, comprising all the classic plant GSTs, such as the relatively well characterised major maize GSTs, contain a single intron. The type III GSTs were first identified as auxin-responsive proteins, though were subsequently shown to have GST activity. These GSTs

**Figure 2**




Dendrogram of plant GSTs illustrating sequence diversity. Plant GST sequences, connected by thick lines, clearly form two major phylogenetic groups (types I and III), with an additional two minor groups (types II and IV). Representative human GST sequences, connected by thin lines, illustrate the relationship between plant and mammalian GSTs; two of the mammalian classes, theta and zeta, have plant homologues, but no such plant homologues have yet been identified for the remaining mammalian classes. The dendrogram was constructed using the following protein sequences (Genbank / SWISSProt accession numbers, or other source, in brackets): *A. thaliana* GST PM239X14 (AT PM239X1; P42769), Maize GST III (ZM GST3; Patent publication No. WO 96/23072 A1), Wheat GST A1 (TA GST1; P30110), Maize GST IV (ZM GST4; P46420), Maize GST I (ZM GST1; P12653), *A. thaliana* ERD13 (AT ERD13; P42761), *Petunia hybrida* GST (PH GST; Y07721), *A. thaliana* GST PM24 (AT PM24; P46422), *A. thaliana* ERD11 (AT ERD11; P42760), Tobacco parB (NT parB; P30109), *Silene cucubalus* GST (SC GST; Q04522), *Solanum commersonii* GST (SoC GST; AF002692), *Hysoscyamus muticus* GST (HM GST; P46423), Maize Bronze2 GST (ZM Bz2; U14599), *A. thaliana* BAC T7N9, gene 15 (AT T7N9 15; AC000348), *Triticum tauschii* GST (TT GST1; AF004358), Tobacco Nt103 (NT 103; Q03664), Potato PRP1 (ST PRP1; P32111), Soybean GST1 (GM GST1; P32110), *Vigna radiata* clone MII-4 (VR MII-4; U20809), *A. thaliana* GST5 (AT GST5; P46421), Maize GST V (ZM GST5; Y12862), *Eucalyptus globulus* auxin-induced protein (EG PAR; U80615), Tobacco parC (NT parC; P49322), Soybean GST2 (GM GST2; Y10820), *Carica papaya* GST (CP GST; AJ000923), Tobacco parA (NT parA; P25317), Soybean glyoxalase I (GM Glyox; P46417), Tobacco C-7 protein (NT C7; X64399), Wheat GST (TA GST T2; AF002211), Carnation SR8 (DC SR8; P28342) and *A. thaliana* mammalian theta-like GST (AT GST TH; AB010072). In addition the following human sequences, representing the major mammalian GST classes are included: alpha class (HS ALPHA; S49975), mu class (HS MU; X08020), pi class (HS PI; X06547), theta class (HS THETA; X79389) and zeta class (HS ZETA; U86529).

have deduced polypeptide sequences which are highly divergent from type I isoenzymes, and the respective genes, where examined, contain two introns. In recognition of the substantial differences between types I and III GSTs they have been recently reclassified, with the type I isoenzymes remaining in the theta class while the type III GSTs have been grouped into a new tau class [24]. Interestingly, while type I GSTs are more abundant than type III GSTs in maize [15,25], type III GSTs predominate in wheat [16,26] and apparently in most dicotyledenous species [14••], though the significance of this difference in GST expression is not known.

In Droog's original classification a further type II GST group was tentatively classified, composed of a limited number of almost identical sequences from carnation which were associated with GST activity [23]. The genes encoding these type II GSTs contained 9 introns. Recently the sequence of another type II enzyme has been identified in wheat (GenBank AF002211), providing further support that these GSTs are members of a discreet type. Intriguingly, mammalian homologues have also been identified with a similar intron structure, and this group of GSTs has been termed zeta class [27].

Although Droog's recent re-classification of GSTs has undoubtedly been useful, its usage of tau and theta terminology may be limiting. Database searches by the authors for plant DNA sequences with homology to mammalian GSTs have identified a putative GST gene in an *A. thaliana* genomic sequence (GenBank AB010072) which is more similar to mammalian theta class GSTs than to any other plant GST. Two expressed sequence tags ESTs corresponding to this theta GST were also identified (GenBank Z35742 and T46668), confirming that the gene was expressed and enabling intron positions to be confidently located and the peptide sequence to be deduced. While mammalian theta GSTs have four introns, this *A. thaliana* sequence has five introns, of which two are located in an identical position to those of mammalian theta GST genes. It is currently uncertain whether the *A. thaliana* sequence is similar enough to mammalian theta GSTs to be placed into the same class, or whether it should be the first member of a new class. We have, therefore, temporarily classified it as a type IV plant GST.

### The structure of plant GSTs

The three-dimensional structure of a plant GST was first elucidated for a type I GST from *A. thaliana* in 1996 [28••]. More recently two maize GSTs involved in herbicide metabolism, GST I (*Zm* GST I-I) and GST III (*Zm* GST III-III), which are both type I GSTs, have also been crystallised and their structures solved [29••,30••]. Despite the low sequence homology between these plant GSTs and mammalian GSTs they share a considerable structural similarity, especially within the amino-terminal domain which forms the glutathione-binding site (G-site). The GSTs from plants were found to have a larger cleft for binding hydrophobic co-substrates than mammalian GSTs and could potentially accept larger and more varied substrates. The structure for maize GST I [29••], when crystallised with the ligand *S*-lactoylglutathione, as compared with that for maize GST III, crystallised without ligand [30••], showed differences in the relative position of a 10-residue loop at the active site. This has been interpreted as evidence for an induced-fit mechanism, where binding of substrate to the GST induces a change in enzyme conformation to better accommodate the substrate, similar to that proposed for mammalian pi class enzymes. Also, GST III was found to have a hydrophobic, highly flexible carboxy-terminal region which may form a lid over the active site on substrate binding to maintain a hydrophobic environment.

Interestingly, the crystal structure of the plant GSTs has revealed some interesting differences as compared with mammalian GSTs. Firstly, the plant GSTs use a serine residue in place of a tyrosine at the active site to activate glutathione for conjugation reactions. Secondly, in the plant GSTs the G-site of each subunit is discreet and self-contained and thus thiol binding is possible without the strict co-alignment of subunits required with mammalian GSTs, where the G-site for each subunit requires an additional residue from the dimer's other subunit [31••]. Thus, much more variation should be tolerated at the dimer interface in plant GSTs and this indeed seems to be the case, with considerable sequence diversity found between the amino acid residues which form the dimer interface.

### GSTs and xenobiotic metabolism

The activities of type I GSTs from maize toward xenobiotic substrate, notably herbicides, has been recognised for some time. Recently we have extended these studies and suggested a rational description of the type I maize (*Zm*) GSTs on the basis of their subunit composition and the nomenclature adopted with mammalian GSTs. Thus, the three major 29 kDa, 27 kDa and 26 kDa subunits have been termed the *Zm* GST I, *Zm* GST II and *Zm* GST III subunits respectively and these can dimerise together to form the *Zm* GST I-I, *Zm* GST I-II, *Zm* GST I-III, *Zm* GST II-II and *Zm* GST III-III isoenzymes [15]. These GSTs have overlapping but definable substrate specificities, with the *Zm* GST I subunit conferring broad ranging conjugating activities toward diverse substrates (Table 1), while the *Zm* GST II and *Zm* GST III subunits showed relatively greater activity toward chloroacetanilide herbicides [15]. The importance of the correct dimerisation of these GSTs in herbicide metabolism has recently been reported, with maize mutants that were unable to form dimers using the *Zm* GST I and *Zm* GST II subunits being less tolerant to the chloroacetanilide alachlor [32]. Interestingly, recent studies have revealed that these GSTs in maize cannot solely be considered to be herbicide detoxifying as they are also able to bioactivate a range of thiadiazolidine herbicides by isomerising them

to the corresponding triazolidines, which are active as protoporphyrinogen oxidase (a key enzyme in chlorophyll synthesis) inhibitors [33,34].

**Table 1**

**Comparison of activity of purified type I maize GST I (*Zm* GST I-I; unpublished data), type III maize GST V (*Zm* GST V-V) [25] and type III soybean GST *Gm*1-1 (GST *Gm*1-1) [35].**

| Substrate | *Zm* GST I-I | *Zm* GST V-V | GST *Gm*1-1 |
|---|---|---|---|
| 2-Chloro-2,4-dinitrobenzene | 1690 | 91 | 122 |
| Ethacrynic acid | 27.0 | 4.9 | 3.7 |
| *p*-Nitrobenzyl chloride | 58.2 | 3.0 | 0.9 |
| Benzyl isothiocyanate | 43.7 | 4.3 | 31.8 |
| 4-Vinylpyridine | NT | 1.0 | 0.5 |
| Metalochlor | 0.04 | 0.25 | 0.23 |
| Flurodifen | 0.01 | 0.52 | 0.40 |
| Cumene hydroperoxide | ND | 0.14* | 0.44* |

*Glutathione peroxidase activity measured as absorbance change at 366 mm/min/mg protein. ND, no activity detected. NT, activity not tested.

In addition to type I GSTs, which have a well defined role in herbicide metabolism, it has recently also become apparent that type III GSTs are able to detoxify xenobiotics. In maize, two type III subunits were identified, *Zm* GST V and *Zm* GST VI, which dimerised together to form the *Zm* GST V-V and *Zm* GST V-VI isoenzymes [25]. Both these GSTs were able to detoxify a range of xenobiotics, and showed unusually high activities toward diphenyl ether herbicides (Table 1). In wheat, all the major GSTs with activities toward herbicides were recognised by an antiserum raised to *Zm* GST V-VI and appeared to be type III GSTs [16]. The involvement of type III GSTs in the metabolism of the chloroacetanilide herbicide dimethenamid in wheat has also recently been demonstrated [26]. Similarly a type III GST from soybean, GST *Gm*1-1, was also active in detoxifying a range of xenobiotics (Table 1), including several herbicides used selectively in this crop [35]. The conjugating activities of GST *Gm*1-1 were of particular interest as they showed substrate-specific binding preferences for specific thiol co-substrates [35]. With most xenobiotics glutathione was the preferred thiol, but with the selective diphenyl ether herbicides acifluorfen and fomesafen and the sulphonyl urea chlorimuron ethyl, homoglutathione, the predominant thiol found in soybean, was the preferred co-substrate. Additional studies have suggested that this thiol preference could explain the selectivity of these herbicides in soybean [12]. In addition to crops, competing weeds have also been shown to contain GSTs active in detoxifying herbicides [11,12]. In black-grass, which is a problem weed in wheat, black-grass GSTs were related to those in the wheat crop, with several GST subunits recognised by an antiserum raised against type III wheat GSTs [36]. Interestingly, herbicide-resistant black-grass populations contained GSTs which were absent in herbicide-susceptible populations and these resistance-specific GSTs showed activity towards several important herbicides [37].

Another issue of recent interest concerning the importance of GSTs in herbicide metabolism has been the induction of type I and type III GSTs by herbicide safeners, compounds which increase the tolerance of cereal crops to herbicides apparently by enhancing their capacity to induce herbicide-detoxifying enzymes such as GSTs. The selective enhancement of the *Zm* GST II subunit in maize by safener application has been recognised for some time, with recent studies confirming that the other two type I GST subunits *Zm* GST I and *Zm* GST III are not similarly responsive [25]. Type III GSTs also seem to be responsive to safener treatment, with the *Zm* GST V subunit being selectively induced by the safener dichlormid, but not by other treatments [25]. Similarly, type III GSTs are the major safener-inducible GST isoenzymes in hexaploid wheat [26] and its diploid progenitor *Triticum tauschii* [37].

Recent enzyme activity data — obtained with pure recombinant GSTs from plants — have allowed comparison of GST activities between type I and III GSTs (Table 1). The data show that GST *Gm* I-I and *Zm* GST V-V, both type III enzymes, have very similar activities, while the type I enzyme *Zm* GST I-I has very different substrate specificity, showing much higher activity towards xenobiotics such as 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid and *p*-nitrobenzyl chloride, but much lower activity towards the herbicides metolachlor and fluorodifen. It is, therefore, tempting to suggest that these two types of plant GST have differing substrate specificities, with GSTs within a type having similar substrate specificities. This, however, may not be the case, as the type I GST *Zm* GST II-II, also known as GST IV, has negligible activity towards CDNB and high activity towards metolachlor and fluorodifen [38], thus being more like a type III GST such as *Zm* GST V-V rather than a type I GST such as *Zm* GST I-I. Further substrate specificity studies on a wider range of plant GSTs should ascertain whether there are any links between enzyme activity and classification, as seems to be the case for mammalian GSTs [14••].

### Roles of plant GSTs in the conjugation of natural products

Although the role of GSTs in the conjugation of xenobiotics in plants is well established, with the *S*-linked peptide glutathione derivative and related metabolites accumulating in the vacuole [20•,22], a similar role in the detoxification of endogenous metabolites has yet to be determined.

Very few natural products have been described which show evidence of glutathione conjugation in the course of their metabolism. This suggests that either natural products are not glutathione conjugated, or that such conjugation is reversible. To date, the best evidence that GSTs are involved in the metabolism of natural

products stems from the observation that the *Bronze2* gene from maize encodes an enzyme showing GST activity [14••]. The Bronze2 GST is integrally involved in the deposition of anthocyanin pigments into the vacuole. It has also been proposed that this GST is involved in the vacuolar targeting of other flavonoids as well as a diverse range of plant hormones [14••]. Thus, in a recent study it was demonstrated that when the isoflavonoid phytoalexin medicarpin was pre-incubated with glutathione and a maize GST preparation, medicarpin was actively imported into mung bean vacuoles [39]. The proposal that these natural products are glutathione conjugated by the Bronze2 GST, or related GSTs [14••], for vacuolar deposition initially seems very plausible, especially in view of the presence of the ABC glutathione conjugate transporter system in the tonoplast membrane [20•,21].

On closer scrutiny, however, there are some problems with this model. Firstly, although many of the auxins and flavonoid derivatives are well known inhibitors of GSTs they have not been reported to undergo glutathione conjugation, nor do they contain suitable electrophilic centres for addition or substitution reactions with the thiol. Secondly, the glutathione conjugates formed from these natural products by the action of GSTs have not been definitively reported. Thirdly, glutathione conjugates, or related derivatives, of these natural products have not been described as accumulating in the vacuole, or elsewhere in the plant. Collectively these observations suggest that the Bronze2 GST is unlikely to catalyse the formation of the type of glutathione *S*-linked conjugates observed in the course of xenobiotic metabolism. Instead, these GSTs may be serving as molecular chaperonins, binding and presenting the natural products to the vacuolar transporter where they are transported in the presence of glutathione. Binding of multiple hydrophobic substrates is certainly suggested by the crystallographic studies showing a large hydrophobic binding cleft on type I GSTs [28••–30••].

Although there is no evidence that any natural products are glutathione conjugated in plants, recent studies have confirmed that stress-inducible GSTs from maize [25], wheat [16] and soybean [35] can detoxify alkenal derivatives which resemble endogenous metabolites arising from oxidative injury. These studies have also shown that these GSTs can detoxify isothiocyanates which are natural allelochemicals in *Brassica* species. Until recently it was believed that phenylpropanoids, such as cinnamic acid and coumaric acid, could serve as GST substrates, with a monomeric 30 kDa protein from maize identified which catalysed the addition of glutathione to the olefinic bond [40]. Recent work, however, has shown that this enzyme is an ascorbate peroxidase [41•], probably acting by indirectly generating thiyl free radicals of glutathione which can then react spontaneously with the alkyl double bond of cinnamic acid to give the observed glutathione conjugate.

## Glutathione peroxidases

Complementary to its function in detoxification through conjugation, glutathione serves an additional protective role in reducing cytotoxic hydroperoxides, which arise as a result of oxidative stress, to the respective alcohols. These reductions are catalysed by glutathione peroxidases (GPOXs):

$$R\text{-}OOH + 2GSH \rightarrow ROH + H_2O + GSSG.$$

Such hydroperoxides include hydrogen peroxide, and hydroperoxides of fatty acids and phospholipids. The GPOXs in plants can be divided into three distinct types of enzyme. These are the selenium-dependent GPOXs composed of four 16 kDa subunits and identified in *Aloe vera* [42], the non-selenium dependent phospholipid hydroperoxide glutathione peroxidases (PHGPXs), and glutathione transferases showing glutathione peroxidase activity (GST-GPOX) [43••]. The tetrameric selenium-dependent GPOX appears to be similar to mammalian cytosolic GPOX. In contrast, PHGPX differs from its mammalian homologue by containing cysteine, rather than selenocysteine at the catalytic site. This enzyme is able to reduce hydrogen peroxide and phospholipid hydroperoxides [43••]. Genes homologous to PHGPX have been cloned from a number of plant sources, including *Nicotiana sylvestris* (P30708), *Avena fatua* (U20000), *Brassica rapa* (L33515, L33544), *A. thaliana* (X89866, AJ000469, AJ000470, AB001568), *Oryza sativa* (D49202) and *Pisum sativum* (AJ000508). Analysis of the PHGPX sequences from *A. thaliana* (X89866 and L33544) suggested that they might be targeted to plastids as well as the cytosol [43••]. Targeting of PHGPX to the chloroplast has recently been confirmed *in vitro* [44]. PHGPX activities are responsive to a wide range of stress treatments which impose oxidative stress [45], with expression also being enhanced in catalase-deficient tobacco [46] and in plants showing antisense-reduced activities of uroporphyrinogen decarboxylase or coproporphyrin oxidase [47]. These observations have led to the proposal that as well as scavenging hydrogen peroxide, GPOXs also serve to detoxify products of lipid peroxidation which may be formed due to the activity of active oxygen species [43••].

### GSTs showing glutathione peroxidase activity

Several plant GSTs have recently been demonstrated to have additional activities as GPOXs, an activity characteristic of mammalian theta class and zeta class GSTs [27]. Although able to reduce a diverse range of organic hydroperoxides GST-GPOXs reportedly differ from the other GPOXs in showing no activity toward phospholipid hydroperoxides and hydrogen peroxide [43••]. Type I and type III GSTs with GST-GPOX activities have been identified in purified isoenzymes from *A. thaliana* [43••], wheat [16], peas [48], maize [15,25] and soybean [35]. In soybean, maize and wheat, the expression of these GST-GPOXs is strongly enhanced by a variety of chemical treatments, particularly those associated

with the formation of active oxygen species. These GST-GPOXs, therefore, may be responding to oxidative stress. GSTs induced by herbicide safeners in both maize and wheat also exhibit GPOX activity, but constitutively expressed GST isoenzymes show lower GPOX activities [15,16,25] — thus, is possible that safeners induce GST expression by mimicking oxidative insult. Overexpression of the type III GST-GPOX Nt107 in transgenic tobacco [49••,50] resulted in seedlings which were tolerant of chilling or salt stress and showed reduced levels of lipid peroxidation under normal and stress conditions. It was suggested that in addition to the direct protective effect of the GPOX activity that this enhanced tolerance may be due to the GPOX-mediated increase in GSSG in the plant cells which then acted as a signal to activate further protective stress responses.

## Conclusions

Surprisingly little evidence is available to definitively identify the role of glutathione and glutathione-dependent enzymes in endogenous plant metabolism. Although plants possess diverse glutathione-dependent enzymes such as formaldehyde dehydrogenases, glyoxalases, glutathione transferases and glutathione peroxidases, the functioning of these systems *in vivo* is only just being addressed. This is most apparent with the glutathione transferases, where there is little evidence that they have any activity in catalysing glutathione conjugation during the course of endogenous metabolism. It is, therefore, tempting to speculate that GSTs do not typically act as transferases, but could instead be functioning as binding and transport proteins for biologically active molecules such as auxins or tetrapyrroles or catalysing alternative reactions such as isomerisations or hydrolysis reactions (Figure 1). In support of this proposal, it has recently been demonstrated that the human zeta class GST is a homologue of *Aspergillus nidulans* maleylacetoacetate isomerase, an enzyme involved in phenylalanine catabolism, and possesses this isomerase activity [51]; this may also be true of plant zeta class (type II) GSTs. It will now be interesting to see how the literature develops as mutant and transgenic plants showing modified expression of glutathione-dependent enzymes are analysed for alterations in metabolism and stress tolerance.

## Acknowledgements

The authors wish to acknowledge funding from BBSRC and Rhône-Poulenc Agriculture Ltd. for the authors' work mentioned in this review.

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