THE FUNCTIONS AND
REGULATION OF GLUTATHIONE
S-TRANSFERASES IN PLANTS

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ABSTRACT
Glutathione S-transferases (GSTs) play roles in both normal cellular metabolism as well as in the detoxification of a wide variety of xenobiotic compounds, and they have been intensively studied with regard to herbicide detoxification in plants. A newly discovered plant GST subclass has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress, and heavy-metal toxicity. In addition, plant GSTs play a role in the cellular response to auxins and during the normal metabolism of plant secondary products like anthocyanins and cinnamic acid. This review presents the current knowledge about the functions of GSTs in regard to both herbicides and endogenous substrates. The catalytic mechanism of GST activity as well as the fate of glutathione S-conjugates are reviewed. Finally, a summary of what is known about the gene structure and regulation of plant GSTs is presented.

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Glutathione S-transferases (GSTs, E.C. 2.5.1.18) are enzymes that catalyze the conjugation of the tripeptide glutathione (GSH) to a variety of hydrophobic, electrophilic, and usually cytotoxic substrates (see 88, 110). GSTs have been found in virtually all organisms, with the first identification of corn GSTs over 25 years ago (40, 128, 130). Plant GSTs were first identified and have been intensively studied because of their ability to detoxify herbicides, and individual GSTs conferring herbicide tolerance have been characterized from most major crop species. Recently, another plant GST subclass has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress, and heavy-metal toxicity. In addition, plant GSTs play a role in the cellular response to auxins and during the normal metabolism of plant secondary products like anthocyanins and cinnamic acid.

The subject of plant GSTs has not been reviewed in this series in the 25 years in which plant GSTs have been studied. However, this first review is quite timely: In the past three years, a vast amount of new information concerning plant GSTs has appeared. The scope of this review encompasses the current knowledge about the enzymatic activity of GSTs toward both herbicides and endogenous substrates, and the review evaluates the very recent data concerning the roles of GSTs in response to auxin, oxidative stress, lipid peroxidation, and in defense against pathogens.
FUNCTIONS OF GSTs IN CELLS: VARIATIONS ON A BASIC THEME

GSTs are most often thought of as detoxification enzymes and indeed were first discovered for their ability to metabolize a wide variety of toxic exogenous compounds (xenobiotics) via GSH conjugation (see 88). However, GSTs have since been found to function in numerous cellular processes that, despite the apparent diversity, all have in common a more basic theme—the recognition and transport of a broad spectrum of reactive electrophilic compounds, regardless of whether the compound is of an exogenous “xenobiotic” origin or of an endogenous “natural” origin.

Detoxification by GSH Conjugation

A key role played by GSTs is their ability to inactivate toxic compounds. An astonishing array of toxic defense compounds are produced by organisms in competition with one another to survive (137). For example, plants synthesize a wide variety of complex secondary products such as phytoalexins, opiates, and flavones, which function as a defense against herbivores and pathogens. Many insects secrete the bitter-tasting alkaloids they obtain from feeding on plants to deter predation from birds or other animals. The modern chemical industry has developed numerous herbicides, insecticides, and synthetic drugs to control certain so-called “pests” in our environment.

The ability to detoxify such harmful compounds is crucial to the survival of cells and organisms. Most organisms have countered exposure to toxic chemicals with the development of detoxification systems to transform, metabolize, and eliminate such compounds from tissues. Remarkably, a common pathway exists in most organisms for the detoxification of electrophilic compounds, which is mediated by three groups of enzymes (63, 119). Phase I (transformation) enzymes such as cytochrome P450 monooxygenases introduce functional groups onto substrates. Phase II (conjugation) enzymes such as UDP-glucosyltransferases and GSTs utilize the functional group as a site of further conjugation, usually resulting in a less toxic and more water-soluble conjugate. Most organisms encode multiple isoforms of both phase I and phase II enzymes, each with their own substrate specificity. Phase III (compartmentation) enzymes, ATP-dependent membrane pumps, recognize and transfer conjugates across membranes for excretion or sequestration. In both plants and animals, the glutathione pump recognizes glutathione S-conjugates for transfer across membranes; in animals, this allows for their excretion from the body (62, 63, 88, 119, 120). Plants have no excretion system, and instead, glutathione S-conjugates are either sequestered in the vacuole or transferred to the apoplast, processes termed “storage excretion” (76, 80, 93, 119, 120).

It should be stressed that these “detoxification enzymes” are remarkably versatile in the recognition of substrates. In plants, members of these same
enzyme families also play normal, defined roles in secondary metabolism and thus recognize and transport both natural and xenobiotic substrates (119). For example, both herbicide metabolism as well as anthocyanin biosynthesis require the coordinate activities of various isoforms of cytochrome P450s, GSTs, and the glutathione pump (see 90, 119, and references therein).

**Targeting for Transmembrane Transport**

GSH conjugation “tags” numerous endogenous substrates in addition to xenobiotic substrates for recognition by the glutathione pump. GSH conjugation is an essential step in the biosynthesis of compounds like leukotrienes in animals; their extracellular biological activity requires transport by the glutathione pump (63, 118, 134). In plants, many secondary metabolites are phytotoxic, even to the cells that produce them, and thus targeting to the appropriate cellular localization, usually the vacuole, is crucial (94, 119, 120). Anthocyanin pigments require GSH conjugation for transport into the vacuole; inappropriate cytoplasmic retention of the pigment not only prevents the production of anthocyanins but is also toxic to cells (see 90 and references therein).

**Protection of Tissues from Oxidative Damage**

The endogenous products of oxidative damage initiated by hydroxyl radicals are highly cytotoxic. This includes membrane lipid peroxides, such as 4-hydroxyalkenals, as well as products of oxidative DNA degradation, such as base propanols. Plant and animal GSTs conjugate GSH with such endogenously produced electrophiles, which results in their detoxification (4, 6, 19, 35, 88, 110, 148). Some GSTs also function as glutathione peroxidases to detoxify such products directly (4, 148, 164).

**Ligandins: Nonenzymatic Binding and Intracellular Transport**

In addition to their catalytic function, GSTs serve as nonenzymatic carrier proteins (ligandins) involved in the intracellular transport of steroids, bilirubin, heme, and bile salts in animal cells (71, 84, 85). Compounds that bind GSTs as nonsubstrate ligands do so at a site other than the catalytic site of the enzyme. In plants, some GSTs apparently serve as carriers of the natural auxin indole-3-acetic acid (IAA), as at least two research groups have identified active GSTs as auxin-binding protein without detecting the formation of IAA-GSH conjugates (8, 68). This nonenzymatic binding may allow temporary storage or modulation of IAA activity or IAA uptake from membranes and trafficking to receptors (8, 68). It has been proposed that the ligandin function of GSTs prevents cytotoxic events that could result from the excessive accumulation of molecules at membranes or within cells (84).
GSTs: EVOLUTION AND CATALYTIC MECHANISM

Catalytic Mechanism of GSTs

GSTs catalyze the nucleophilic attack of the sulfur atom of glutathione (GSH, γ-glutamylcysteinylglycine) to the electrophilic center of a wide variety of substrates (see 88, 110). GSTs typically function as either heterodimers or homodimers of subunits with molecular weights in the range of 23 and 29, though microsomal GSTs in animals are trimeric or tetrameric and have subunits of ~14 kD (26, 102), and a maize GST with activity against phenylpropanoids functions as a 30-kD monomer (21). Each subunit has a GSH binding site (G-site) and an adjacent electrophilic substrate binding site (H-site) (88, 116). Substrate specificity of the G-site is high, with only GSH and structurally related molecules serving as substrates (21, 80, 88, 116). In contrast, GSTs have a broad specificity for the electrophilic substrate (Figure 1). Talalay (147) has suggested that compounds that induce GSTs or that are recognized as substrates share a common chemical signal—carbon-carbon double bonds adjacent to an electron-withdrawing group (Figure 1). This feature, termed a “Michael acceptor,” is either contained naturally by GST substrates or acquired by phase I metabolism (147).

GSTs are products of gene superfamilies, each producing isozymes with broad substrate specificities. Despite the fact that they perform a similar reaction, GSTs share very little overall amino acid sequence identity, typically no more than 25–35%, though there are usually regions of much higher localized similarity within the N-terminus (34, 88). Given the array of compounds recognized as GST substrates, sequence diversity is to be expected. Well-conserved amino acids are likely to be important in common functions, i.e. GSH binding or catalysis. X-ray crystallography and site-directed mutagenesis demonstrate that a highly conserved N-terminal arginine residue is involved in GSH binding at the G-site, and an N-terminal tyrosine residue facilitates formation of the thiolate (GS-) ion (see 82, 109, 113, and references therein). Specific glutamine, proline, histidine, and aspartic acid residues bind GSH or maintain enzyme structure (see 82, 110, 113, and references therein). Site-directed mutagenesis or X-ray crystallographic structures of plant GSTs have not yet been reported, although plant GSTs also possess the well-conserved N-terminal arginine residues (34).

The standard experimental assay for GST activity utilizes 1-chloro-2,4-dinitrobenzene (CDNB, Figure 1), a model substrate for most, but not all, GSTs. Conjugation of CDNB with GSH (by chlorosubstitution) results in a change in absorbance of the compound at 340 nm, providing a simple spectrophotometric assay (89). Not all GSTs can use CDNB as a substrate, and thus the activity of certain GSTs may be underestimated or even undetected when using this assay.
Evolution of Glutathione and GSTs

GSH and GSTs are widely distributed in aerobic organisms and are hypothesized to have evolved in aerobic bacteria for their ability to prevent oxygen toxicity (39, 88, 109). GSH is synthesized from amino acids by the sequential action of γ-glutamyl cysteine synthetase and glutathione synthetase (1). In addition to serving as the thiol substrate of GSTs, GSH is a substrate of glutathione peroxidase and glutathione reductase (1, 98, 114) and in plants is a precursor to phytochelatins, heavy-metal binding proteins (112, 135).

In mammals, GST subunits are classified into alpha, mu, pi, sigma, and theta classes on the basis of amino acid identity, immuno-crossreactivity, and substrate specificity (12, 87). The theta class is evolutionarily the most ancient group and is found in vertebrates, Drosophila, plants, and Methylobacterium (88, 109). This suggests that the theta class is representative of a progenitor GST that arose before the divergence of prokaryotes and eukaryotes and that these GSTs may still function in the detoxification of reactive oxidation prod-

\[ \text{Figure 1} \quad \text{Compounds that are substrates of GSTs, induce GST gene expression, or bind GST noncatalytically: (a) CDNB: 1-chloro-2,4-dinitobenzene, a model substrate of most GSTs used to experimentally assay GST activity; (b) examples of Michael acceptor structure, where } Z = \text{ electron withdrawing groups such as } \text{NO}_2, \text{CHO}, \text{COCH}_3, \text{CN}, \text{CONH}_2 \text{ (147); (c) IAA: indole-3-acetic acid, an inducer; (d) } \alpha\text{-NAA: } \alpha\text{-naphthylacetic acid, an inducer; (e) } 2,4\text{-D: 2,4-dichlorophenoxyacetic acid, an inducer; (f) atrazine, an S-triazine herbicide and GST substrate; (g) metolachlor, a chloroacetamide herbicide and known GST substrate; (h) cinnamic acid, a substrate; (i) SA: salicylic acid, an inducer; (j) cyanidin-3-glucoside, a substrate of the } b\text{-2 GST; (k) thymidine propanol, a toxic product of oxidative DNA degradation and substrate of animal GSTs; (l) 4-hydroxyalkenal, a toxic product of lipid peroxidation and substrate of animal GSTs.} \]
All plant GSTs studied thus far are most similar to the theta class, although a carnation GST also shares similarities in sequence and intron position to mammalian alpha class GSTs, suggesting that the evolution of the alpha class GSTs may have begun before the plant and animal kingdoms diverged (65). The emergence of the alpha, mu, and pi GST classes may have provided animals and fungi with new detoxification enzymes to counter defense compounds produced by plants (109). Gene duplication, followed by exon shuffling, of an ancestral GSH binding protein may have been a mechanism that generated the different catalytic properties of the members of the GST superfamily (88).

CLASSES OF PLANT GSTs

Although all known plant GSTs are most similar to the theta class, they can be further classified into several subgroups, as proposed recently by Droog et al (32). This classification is attractive in that it is based on amino acid sequence identity and conservation of intron:exon placement and not on substrate recognition or antibody crossreactivity, features that are far more difficult to fully characterize. A phylogenetic tree of plant GSTs is shown in Figure 2. The only GSTs that are not classified are those for which sequence information is not yet available, and several more subgroups may be discovered as more sequences become available. For some of the genes in each subgroup, the intron:exon structure has not been determined either because a cDNA was cloned or because the gene was only partially sequenced, and thus placement in the group has been by amino acid sequence alone. As yet, there is no unified

Figure 2  Phylogenetic tree of plant type I, II, and III GSTs. The tree was constructed using the DNASTAR sequence program. The sequences aligned are listed at the right hand of the figure and were obtained by retrieval of protein sequences from Genbank or PIR as listed in Table 1.
Table 1  Plant GSTs

<table>
<thead>
<tr>
<th>Type I GSTs</th>
<th>Plant</th>
<th>Protein</th>
<th>Amino acids per subunit</th>
<th>Substrates</th>
<th>Protein or mRNA induced by</th>
<th>Database accession number</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>GST I</td>
<td>Constitutive homodimer, 29 kD</td>
<td>227</td>
<td>alachlor, atrazine, CDNB</td>
<td>flurazone, dichlorimid</td>
<td>X076755</td>
<td>first published sequence of a plant GST</td>
<td>47, 100, 103, 125, 163</td>
</tr>
<tr>
<td></td>
<td>GST II</td>
<td>Heterodimer; 27-kD subunit-inducible, 29-kD subunit-constitutive</td>
<td>223 (27 kD), 227 as (29 kD)</td>
<td>alachlor, CDNB</td>
<td>flurazone, dichlorimid</td>
<td>U12679 (27 kD)</td>
<td>not induced by heat, drought, chilling, or pathogens</td>
<td>11, 56, 67, 163</td>
</tr>
<tr>
<td></td>
<td>GST III</td>
<td>Constitutive homodimer, 26 kD</td>
<td>222</td>
<td>alachlor, metolachlor, CDNB</td>
<td>dichlorimid</td>
<td>X04375, X04453</td>
<td></td>
<td>47, 100, 107, 125</td>
</tr>
<tr>
<td></td>
<td>GST IV</td>
<td>Inducible homodimer, 27 kD</td>
<td>223</td>
<td>chloroacetamide and S-triazines, metolachlor</td>
<td></td>
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<td></td>
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<tr>
<td>Wheat</td>
<td>GST 29, 29 kD</td>
<td></td>
<td>229</td>
<td>CDNB (weak)</td>
<td>Transcripts induced over 20X with pathogen attack; induced by GSH</td>
<td>X56012</td>
<td>involved in onset of induced resistance to powdery mildew; not induced by xenobiotics</td>
<td>35, 95</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>PMA239X14</td>
<td>Constitutive 25-kD GST and glutathione peroxidase</td>
<td>218</td>
<td>CDNB (weak); high affinity for lipid peroxides</td>
<td>developmentally downregulated</td>
<td>X68304</td>
<td>not induced by wounding, IAA, 2,4-D, methyl jasmonate</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>AW1D4</td>
<td>n.d.</td>
<td>n.d.</td>
<td>mRNA induced by wounding and ethylene; developmentally regulated</td>
<td>n.a.</td>
<td>n.a.</td>
<td>only partially sequenced</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>gst2/Atpm24</td>
<td>Microsomal GST and glutathione peroxidase, ABP, 24 kD</td>
<td>212</td>
<td>cumene hydroperoxide, trans-stilbene oxide, CDNB; binds IAA</td>
<td>mRNA induced by ethylene; developmentally regulated</td>
<td>Atpm24: X75303 gst2: L11601</td>
<td>Cinnamic acid and IAA are not substrates</td>
<td>166</td>
</tr>
<tr>
<td>STP</td>
<td>Size (kD)</td>
<td>Activity</td>
<td>Induction</td>
<td>Expression</td>
<td>Other Info</td>
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<tr>
<td>ERD11</td>
<td>23.5</td>
<td>n.d.</td>
<td>mRNA induced by dehydration stress</td>
<td>D17672</td>
<td>Not induced by 2,4-D, 6-BAP, ABA, GA</td>
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<tr>
<td>ERD13</td>
<td>24.2</td>
<td>n.d.</td>
<td>mRNA induced by dehydration stress</td>
<td>D17673</td>
<td>Not induced by 2,4-D, 6-BAP, ABA, GA</td>
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<tr>
<td>Broccoli</td>
<td>26.5</td>
<td>n.d.</td>
<td>CDNB, 4-nitrophenethyl bimide</td>
<td>n. a.</td>
<td>Only partially sequenced; competitively inhibited by 2,4-D</td>
<td></td>
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</tr>
<tr>
<td>Sugarcane</td>
<td>Heterodimer, 22.5 and 24 kD</td>
<td>n.d.</td>
<td>dichloroiodobenzene, ethacrylic acid, and other electrophilic substrates</td>
<td>n. a.</td>
<td>Only partially sequenced; competitively inhibited by 2,4-D</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Silene cucubalis</td>
<td>Constitutive</td>
<td>213</td>
<td>n.d.</td>
<td>n.d.</td>
<td>cDNA: M84968; genomic: M84969</td>
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<tr>
<td>Hyoscyamus naticus</td>
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<tr>
<td>Hmgst-1</td>
<td>25 kD auxin-binding protein</td>
<td>212</td>
<td>CDNB; also binds IAA and 2,4-D</td>
<td>X78203</td>
<td>Binds IAA at noncatalytic site, 2,4-D at catalytic site</td>
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<td>Type II GSTs</td>
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<tr>
<td>Carnation GST1, (gSR8), GST2</td>
<td>25 kD</td>
<td>220</td>
<td>unknown; speculate lipid peroxides</td>
<td>gSR8: M64268; GST1: L05915; GST2: L05916</td>
<td>Genes are 95% similar in sequence and intron placement, 10 exons, 9 introns</td>
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<td>Type III GSTs</td>
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<tr>
<td>Soybean GmHsp26A or GH2/4</td>
<td>26 kD</td>
<td>225</td>
<td>CDNB</td>
<td>M20363 PIR: A33654</td>
<td>Cadmium induces splicing failure; 14-kD protein produced</td>
<td></td>
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<tr>
<td>Potato prp1-1 (gene), renamed gsr1</td>
<td>26-kD, ABP, and pathogenesis-related protein PRP1-1</td>
<td>217</td>
<td>CDNB; binds IAA</td>
<td>J03679</td>
<td>mRNA not induced by abiotic stimuli (wounding or CdCl2), IAA and lipid peroxides are not substrates</td>
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Table 1  Plant GSTs (continued)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amino acids per subunit</th>
<th>Substrates</th>
<th>Protein or mRNA induced by:</th>
<th>Database accession number</th>
<th>Comments</th>
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<td>Tobacco</td>
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<tr>
<td>parA/NH114</td>
<td>GST 3-1, 25.7 kD</td>
<td>223</td>
<td>CDNB (weak)</td>
<td>mRNA induced by 2,4-D, weakly by IAA and NAA; Cd (no effect on splicing), SA</td>
<td>eDNA: M20274 genomic: D90215 (parA)</td>
<td>Expressed during Go to S mRNA not induced by HS, ABA, cytokinins; localized to nucleus</td>
</tr>
<tr>
<td>parC/NH107</td>
<td>GST 2-1, 25 kD</td>
<td>221</td>
<td>CDNB</td>
<td>mRNA induced by 2,4-D and weakly by IAA and NAA; slightly heat-inducible</td>
<td>NT107; X56266 parC: X64398</td>
<td>not induced by Cd</td>
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<td>NH103</td>
<td>GST 1-1, 25.7 kD</td>
<td>223</td>
<td>CDNB</td>
<td>mRNA induced by 2,4-D and weakly by IAA and NAA</td>
<td>NT103; X56263 GNT1: X56268 GNT35: X56269</td>
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<tr>
<td>Nicotiana plumbaginifolia mut 1 (pLS216)</td>
<td>25 kD</td>
<td>219</td>
<td>n.d.</td>
<td>auxin- and cytokinin-regulated</td>
<td>n.a.</td>
<td></td>
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<td>Maize</td>
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<tr>
<td>Bronze-2</td>
<td>26 kD</td>
<td>241, 78-bp intron</td>
<td>CDNB and natural substrate cyanide-3-glucoside (anthocyanin precursor)</td>
<td>Induced by Cd, ABA, arsenite</td>
<td>U14599 PIR: JQ6987</td>
<td>last gene in anthocyanin biosynthesis; not induced by 2,4-D, HS; Cd induces splicing failure</td>
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<tr>
<td>Arabidopsis thaliana</td>
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<td>GST 5</td>
<td>25.9 kD</td>
<td>224</td>
<td>CDNB; IAA, 2,4-D competitively inhibit enzyme activity, SA noncompetitively</td>
<td>mRNA induced by wounding, HS</td>
<td>D44465</td>
<td>mRNA not induced by IAA</td>
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<tr>
<td>Unclassified GSTs</td>
<td>Sorghum GSTs</td>
<td>Chickpea</td>
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<td></td>
<td>4–5 additional GSTs safrer-induced</td>
<td>Heterodimers, 27 kD and 29 kD</td>
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<tr>
<td></td>
<td>CDNB, metolachlor</td>
<td>oxadiazon oxadiazon-induced n.a.</td>
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<td>Safeners (flurazazole, oxabetranil, naphthalic anhydride) induce 4–5 metolachlor GSTs</td>
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<td>24, 25</td>
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<tr>
<td>Pea</td>
<td>n.d.</td>
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<tr>
<td></td>
<td>37 kD</td>
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<td>n.d.</td>
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<tr>
<td></td>
<td>47 kD</td>
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<td>n.d.</td>
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<td>Protein induced by Cd, antrazine, paraquat, alachlor</td>
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<td>does not have activity with CDNB, metolachlor, atrazine, EPTC sulfoxide or lipid peroxides; can utilize cyst or GSH as thiol substrate</td>
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<td>enzyme crosers with corn GST antibodies</td>
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nomenclature for plant GSTs as there is for animal GSTs (87). A brief summary of the three types of plant GSTs follows; the reader should consult Table 1 for specific information about different properties of each GST.

**Type I**

These GSTs (where the gene structure is known) contain three exons and two introns (Table 1). This group includes the four distinct maize GSTs that differ with respect to subunit composition and substrate specificity toward herbicides (11, 47, 56, 60, 61, 67, 100, 103, 107, 125, 128, 130, 153, 163). Some type I GSTs appear to function as defense genes or cellular protectant genes, producing proteins in response to pathogen attack, wounding, senescence, and the resulting lipid peroxidation that accompanies these processes (4, 35, 72, 168). This is discussed in depth in the section on Oxidative Stress. Other type I GSTs are induced in response to auxins (144, 146) and may serve a ligandin function toward IAA (7, 8, 166). Type I GSTs have also been cloned from *Silene* (75, 111), sugarcane (131), broccoli (86), and *Arabidopsis* (73), though the functions of these GSTs are as yet unclear.

**Type II**

These GSTs contain ten exons and nine introns and have only been reported in carnation (64, 65, 99) as ethylene- and senescence-related genes expressed in floral organs (Table 1). Significant amino acid sequence homology exists with type III GSTs, but the intron:exon patterns are characteristic of mammalian alpha class GSTs.

**Type III**

These GSTs (where the gene structure is known) contain two exons and one intron (Table 1). This subclass was originally identified as a set of homologous genes from a variety of species that were inducible by a range of different treatments—particularly auxin, but also ethylene, pathogen infection, heavy metals, and heat shock (see Table 1). Each successive gene isolated was noted to be involved in stress responses, although the functions of the proteins were not apparent from sequence identity. The genes have alternatively been called multiple stimulus response (*msr*) genes (30), auxin-regulated genes (34, 141), or the auxin-regulated gene (ARG) subgroup (162). Only after several years were these genes recognized to encode GST enzymes.

The first gene, soybean *Gmhs2p26-A* (discovered independently as *GH2/4*), was both heat shock- and auxin-inducible (17, 48) but shared only a slight resemblance to other small heat shock proteins (HSPs) and contained neither a characteristic heat shock element (HSE) or an auxin-regulated element (AuxRE) in the promoter (3, 17, 48). However, the gene was induced by a wide variety of chemicals, including 2,4-dichlorophenoxyacetic acid (2,4-D),...
abscisic acid (ABA), cadmium, and other heavy metals, and intron processing was inhibited by cadmium. The potato prp-1 gene, induced during fungal infection, was noted to be highly similar to Gmhs26-A (150). A number of homologous auxin-regulated tobacco genes were cloned and found to also be induced by a variety of other stress-inducing agents (10, 30, 34, 139, 140, 142, 143, 145, 157). The maize anthocyanin biosynthetic gene Bronze-2 (Bz-2) was next added to this group (104, 123); as with hsp26-A, Bz2 intron processing is inhibited by cadmium (91) and also varies with environmental conditions (104).

Droog et al (34) were the first to report that the tobacco Nt103 protein had substantial GST activity. They proposed that the entire auxin-regulated tobacco gene family, as well as the other homologous genes, may be GSTs, and they renamed the protein GST1-1. This unexpected finding prompted investigators to examine the functions of the other proteins, such that now most of the genes have been shown to encode proteins with GST activity. GST(CDNB) activity was shown for the protein encoded by the GH2/4 gene (156), which is identical to the protein encoded by the Gmhs26-A gene, now renamed GmGST26-A (E Czarnecka, personal communication); the potato PRP1 protein, now renamed GST1 (49); the tobacco Nt107/parC protein, now renamed GST2-1 (32); the Nt114/parA protein, now renamed GST3-1 (32, 140); the Arabidopsis GST5 protein (162); and the maize BZ-2 protein, which was found to also conjugate GSH to cytoplasmic anthocyanin precursors, allowing transport of anthocyanins into the vacuole (90).

At least one type III GST, the parA protein, may be localized to the nucleus (140). parA is homologous to the E. coli stringent starvation protein (SSP) that binds RNA polymerase during nutrient starvation and thus may function in transcriptional regulation during stress (140). Alternatively, parA may function as a GST in the nucleus to detoxify cytotoxic DNA degradation products formed during oxidative stress, which are known to be substrates for mammalian GSTs (6). Although the exact function of parA is unknown, its nuclear localization provides several interesting possibilities.

**Unclassified GSTs**

GST activity has been demonstrated in over 33 plant species (see 80), although in many cases individual GST proteins have not been purified. In some species, individual GSTs have been characterized, but amino acid sequence is not yet available, preventing their being grouped with any of the above classes (Table 1). In wheat, GST25 and GST26 were strongly induced by cadmium, atrazine, paraquat, and alachlor but not by pathogen attack (95). Sorghum, chickpea, and Norway spruce each contain several herbicide-active GSTs (22, 23, 46, 59, 124). In pea, there are both soluble and microsomal GSTs with activity toward herbicide safeners and the natural substrate cinnamic acid (29).
Maize and French bean each contain GSTs with activity toward cinnamic acid and other phenylpropanoid substrates (21, 36).

**HERBICIDE RESISTANCE AND GSTs**

The first known function for plant GSTs was in the metabolism of herbicides to nontoxic forms (40, 128, 130), and intensive research over the last 25 years has resulted in the identification of numerous GSTs conferring tolerance to various herbicides. Characteristics of individual GSTs catalyzing herbicide detoxification, selective induction of GSTs by herbicide safeners, and identification of the genes involved in herbicide metabolism are of great interest for many reasons; this research not only provides the knowledge to selectively manipulate GST activity or herbicide tolerance but can also be extended to provide a framework for understanding the metabolism of endogenous compounds in plants.

**Selective Herbicides**

Numerous synthetic herbicides have been developed to control weeds in important agronomic crops. Several comprehensive reviews on the mechanism of herbicide action have been published (57, 101). Selective herbicides are those that kill various weed species without causing significant damage to crop species. Herbicide selectivity can be partially attributed to differences in herbicide uptake or in the method of application. However, physiological and genetic evidence gathered over the past 25 years clearly indicates that frequently the principle determinant of herbicide selectivity in plants is the ability to metabolize and thus detoxify herbicides. There are many documented cases where the formation of the herbicide-GSH conjugate in the resistant but not in the susceptible species is responsible for herbicide selectivity (44, 74, 76, 81, 107, 127). Several specialized reviews thoroughly discuss this subject (16, 76, 79, 80, 127, 129).

It was first shown in the early 1970s that GSH conjugation is responsible for herbicide detoxification (40, 128, 130). GSTs are not equally distributed among plants, and plants with higher GST activity levels will withstand exposure to herbicides that kill susceptible species (76, 79, 127, 129). For example, maize and sorghum are tolerant to atrazine because they contain high levels of the GST that catalyzes atrazine-GSH conjugation, resulting in the conversion of atrazine to a nontoxic, water-soluble form. Susceptible species (pea, oats, wheat, barley, and many broadleaf weeds) do not have this GST activity. Similarly, GSTs have been shown to be responsible for the tolerance of maize to alachlor (107), metolachlor (22, 45, 61, 159), and EPTC sulfoxide (81). GSTs that catalyze the formation of fluorodifen-GSH conjugates are higher in resistant species (cotton, corn, peanut, pea, soybean, okra) than in susceptible species (tomato, cucumber, squash) (41, 76). Acifluorfen selectivity results
from a more rapid rate of GSH conjugation in soybean than in weed species (42), and high GST levels in sorghum confer tolerance to metolachlor (23, 44, 46).

Even within species, GSTs vary greatly in substrate specificity. To date, at least 6 GST isoforms have been characterized in maize. GSTI (29 kD) and GSTIII (26 kD) are constitutive homodimers with activity against CDNB and alachlor (100, 103, 107, 153). GSTII is a safener-induced heterodimer (27 and 29 kD) also active against CDNB and alachlor (56, 100, 153), and GSTIV is a safener-induced homodimer of 27 kD (identical to the 27-kD subunit of GSTII) active against alachlor and metolachlor but not CDNB (45, 61). The BZ-2 GST (26 kD) is active against CDNB and the anthocyanin precursor cyanidin-3-glucoside (90). The sixth GST is a 30-kD monomer active against phenylpropanoids but not CDNB, metolachlor, or atrazine (21). Variations of individual GSTs between different cultivars can be responsible for differential herbicide tolerance (121). For example, the atrazine-sensitive maize strain GT112 was shown to have less than 1% of the GST activity of the atrazine-resistant strain GT112 RfRf (128).

Herbicide Safeners

The usefulness of some herbicides can be extended by certain chemicals, referred to as herbicide safeners, antidotes, or protectants. Grass crops such as maize and sorghum have little intrinsic tolerance to thiocarbamate (e.g. EPTC) and chloroacetamide herbicides (e.g. metolachlor, alachlor), but pretreatment with safeners such as flurazole, dichlormid, and benoxacor greatly enhances the tolerance to these herbicides by selectively inducing GST activity and, in turn, elevating the rate of herbicide detoxification via GSH conjugation (22, 23, 44, 46, 53, 54, 60, 61, 81, 103, 159, 163). In this way, safeners decrease herbicide injury to crop species without decreasing injury to the weed species. The safeners themselves are not toxic but do exhibit structural similarities to herbicides and appear to act by inducing gene expression (53, 54, 163). In some cases, pretreatment with low doses of an herbicide enhances the capacity of tissues to metabolize the same herbicide during a following exposure, mimicking the effect of a safener (38, 66).

PHENYLPROPANOIDs AS NATURAL SUBSTRATES OF PLANT GSTs

Many plant secondary metabolites are protective compounds that can be toxic not only to herbivores and pathogens but also to the cells that produce them. The enzymes involved in biosynthesis of these compounds—cytochrome P450s, UDP glucosyltransferases, and GSTs—are also the same enzymes of xenobiotic metabolism (119). Recent evidence suggests that naturally synthe-
sized plant metabolites are recognized, transported, and metabolized in similar ways as herbicides and other xenobiotics.

**Anthocyanins and GSTs**

Anthocyanin pigments are synthesized in the cytoplasm but are ultimately localized to the vacuole (58, 94, 160). In maize, mutations in the anthocyanin biosynthetic gene *Bronze-2* impart bronze pigmentation to cells that results from the inappropriate accumulation of the anthocyanin precursor cyanidin-3-glucoside in the cytoplasm, causing localized necrosis, poor vigor, or even death of plants. BZ-2 activity results in the transfer of cyanidin-3-glucoside to the vacuole, where the anthocyanin takes on its deep red or purple color. Recently, BZ-2 was shown to be a GST that catalyzed the formation of anthocyanin-GSH conjugates (90), which allows transport into vacuoles by what appears to be the glutathione pump. Thus, cyanidin-3-glucoside is a natural GST substrate. At least one other maize GST can apparently recognize cyanadin-3-glucoside as a substrate, though only weakly, as some *bz-2* mutant maize ears contain pale pink rather than bronze kernels, in contrast with the deep purple color resulting from BZ-2 action (161).

In *Petunia*, the anthocyanin biosynthetic gene *An13* has recently been cloned and shown to have significant homology with type I GSTs and, to a lesser extent, BZ-2 (E Souer & R Koes, personal communication). Complementation studies have shown that BZ-2 is able to provide the GST activity to restore red pigmentation in *an13* mutant *Petunia* flower sectors, and likewise, *An13* is able to restore purple pigmentation in *bz-2* maize tissues (MR Alfenito, personal communication). In addition, the *GmGST26-A* (GH2/4) GST gene complements the *bz2* mutation, producing purple pigmentation in *bz-2* maize tissues (MR Alfenito, personal communication).

**Phytoalexins, Cinnamic Acid, and GSTs**

One mechanism by which plants defend themselves against microbial infection or fungal attack is through the formation of phytoalexins at infection sites (20). Phytoalexins are synthesized in inclusions only after exposure to elicitors such as cell wall fragments of plants, fungi, or microorganisms (20). Because these inclusions release cytotoxic phytoalexins into the cytoplasm the cell dies, but the growth of bacteria and fungi is inhibited in the process (20). Phytoalexin synthesis in response to fungal elicitors has been shown to be strongly inhibited by cinnamic acid, a precursor to phytoalexins. Glutathione S-cinnamoyl transferases (GCSTs) have been characterized in French bean, pea, and corn (21, 22, 24, 29, 36) and can be induced in response to fungal elicitors (36). GCSTs catalyze conjugation of cinnamic acid with GSH and have been hypothesized to remove inhibitory cinnamic acid during the initial steps of
phytoalexin synthesis (5, 36) and to reduce accumulation of other toxic phenolic compounds produced under stress conditions (21).

**AUXINS AND GSTs**

Many plant GST genes are auxin-inducible; indeed, the type III GSTs are often referred to as auxin-regulated genes (32, 34, 141). However, “auxin” is a generic term for compounds that induce shoot elongation; auxins need only resemble one another in their physiological action and not necessarily in their chemical structure (154). Since the discovery of the natural auxin IAA, numerous synthetic compounds have been developed with similar physiological activity, including α-naphthoxyacetic acid (α-NAA) and the chlorophenoxyacetic acids 2,4-D and 2,4,5-T (see Figure 1). Many synthetic auxins are potent herbicides because they are very stable and not subject to destruction by the IAA-oxidase system (2). 2,4-D is also widely used in research as the auxin component of plant tissue culture media. The next section attempts to clarify the role of GSTs in binding, and perhaps detoxifying, auxins from different sources.

**Some GSTs Are Auxin-Binding Proteins**

In the search for cellular auxin receptors, some auxin-binding proteins (ABPs) have been identified as active GSTs (68). The Hmgst-1 protein from *Hyoscyamus muticus* bound tritiated 5-azido-IAA by photoaffinity labeling and had sequence homology with GSTs and showed GST activity in vitro (7). The *Hmgst-1* cDNA is highly similar to the tobacco type I GST *parB* (8). By examining the ability of different auxins and inactive auxin analogues to competitively inhibit GST(CDNB) activity, Bilang & Sturm (8) proposed that different auxins bind different sites on the GST, with IAA, α-NAA, and indole-3-butyric acid binding to a noncatalytic site and 2,4-D, 2,4,5-T and inactive auxin analogues (2,3-D, 2,4,6-T) binding to a catalytic site.

Other ABPs have also been found to be GSTs. The *Arabidopsis* Atpm24 protein, isolated from plasma membrane vesicles by photoaffinity labeling, had sequence homology to type I GSTs and GST activity in vitro (166). The potato PRP1 protein, a type III GST, also bound 5-azido-IAA (49). A 65-kD ABP has recently been isolated from mung bean with N-terminal amino acid sequence homologous to Hmgst-1 and Atpm24, and also to other type I GSTs (A Jones, personal communication). Homologous genes for the 65-kD protein appear to be present also in *Arabidopsis*, maize, wheat, tomato, *Petunia*, and soybean.

**Possible Functions of GSTs as Auxin-Binding Proteins**

Several possible roles of GSTs as auxin-binding proteins can be envisioned. IAA may bind to GSTs as a nonsubstrate ligand and thus serve a ligandin
function. In animals, binding of nonsubstrate ligands occurs at a site distinct from the catalytic site, usually in the C-terminus (113), and in a similar manner, IAA binding is proposed to occur at a noncatalytic site (8). This noncatalytic binding might allow for temporary storage, transport, or uptake of IAA. The expression of Hmgst-1 in stems, where basipetal IAA movement takes place, would suggest that this GST is involved in intracellular IAA transport (8).

IAA may also be conjugated with GSH, perhaps for modulation of activity, temporary storage as a conjugate, or cellular detoxification. In vivo, IAA is conjugated with amino acids or glucose to escape inactivation by decarboxylation (2, 15, 106). It has been proposed that IAA conjugates are "slow-release" forms of free IAA in plant tissues (51). Many GST substrates are glucosylated before GSH conjugation; however, IAA-GSH or IAA-cys conjugates have not been detected, and the Atpm24 GST and the potato PRP-1 GST were unable to catalyze the formation of IAA-GSH in vitro (49, 166). Nevertheless, the lack of evidence does not eliminate the possibility that IAA-GSH conjugates are formed, but it does indicate that further research must be done to determine the metabolic routes of IAA in vivo.

**Auxins Both Bind GST Proteins and Induce GST Gene Expression**

While many plant GST genes are auxin-inducible, several recent findings have shown that, unlike other auxin-regulated genes such as the SAURs, pGH3, Aux22, Aux28, and pIAA4/5 (see 141), the auxin-regulated GSTs were also induced by inactive auxin analogues and many other compounds with no hormonal activity. The soybean GH2/4 gene is inducible not only by strong auxins (2,4-D; 2,4,5-T; α-NAA) and salicylic acid (SA) but also by numerous other electrophilic compounds, including weak auxins (β-NAA), inactive auxin analogues (2,3-D and 2,3,6-T), and inactive SA analogues (3-hydroxybenzoic acid, 4-hydroxybenzoic acid) (155, 156). The GST(CDNB) activity of the tobacco GST1-1 (Nt103) and GST2-1 (Nt107) was competitively inhibited by 2,4-D but also by compounds that are structural analogues of 2,4-D but that are not active auxins—phenoxyacetic and benzoic acid derivatives containing at least one chlorine atom—and not by the natural plant auxins IAA, indole-3-butyric acid, or by α-NAA (32). The Hmgst1 ABP mRNA was not induced by IAA or α-NAA but was induced by 2,4-D and 2,4,5-T as well as 2,3-D (8).

Thus it is clear that different auxins induce and bind GSTs differently. These differences are not contradictory but indicate that GSTs have several functions in the response to auxins: They not only bind the natural auxin IAA, possibly for regulation of activity, transport within cells, or glutathione conjugation, but also are induced by, and possibly detoxify, numerous electrophilic substrates, some of which have auxin activity. It is very likely that auxins from
various sources can be perceived by cells either as true hormones, binding GSTs as a ligand and inducing expression of some GSTs and other so-called genuine auxin-regulated genes (141), or as electrophilic xenobiotic substances to induce GST gene expression selectively.

OXIDATIVE STRESS AND GSTs

In addition to being inducible by auxins and inactive auxin analogues, many plant GSTs are induced by heavy metals, pathogen attack, wounding, ethylene, and ozone. It has been suggested that a common effect of all the processes is the generation of active oxygen species (AOS) produced during oxidative stress and that the GSTs induced respond to oxidative stress to protect cellular components from damage (83, 151, 155, 156).

Active Oxygen Species

Aerobic organisms unavoidably produce AOS, such as superoxide radicals ($O_2^{-}$), hydroxyl radicals ($OH^•$), and hydrogen peroxide ($H_2O_2$), during oxygen consumption. Under normal conditions, antioxidant defenses like superoxide dismutase (SOD), catalase, peroxidase, ascorbic acid, and glutathione prevent AOS formation or scavenge those already present (1, 55, 98, 114, 122). During oxidative stress, the balance between AOS production and antioxidant scavenging is shifted in favor of AOS production (122). One particularly harmful effect of AOS production is membrane lipid peroxidation, which converts fatty acids into hydrocarbon fragments such as highly cytotoxic 4-hydroxyalkenals (Figure 1) (37, 69, 110). These reactive electrophiles have strong inhibitory effects on enzymes such as adenylate cyclase and inhibit DNA and protein synthesis (19, 37, 69). Other consequences of AOS production include the formation of base propenals, highly cytotoxic products of oxidative DNA damage (Figure 1) (6).

Sources of AOS in Plants

PATHOGEN ATTACK, SALICYLIC ACID, AND $H_2O_2$ Pathogen attack in plants rapidly initiates a variety of defense responses, termed the hypersensitive response (HR). During this process, AOS are produced, rather than destroyed, to kill or damage the invading pathogen; within 2–3 min of infection there is a rapid and transient generation of $H_2O_2$ known as the oxidative burst (13, 35, 83, 96, 115, 136, 151, 152). The $H_2O_2$ generated serves to reinforce cell walls, restricting fungal growth, and at higher concentrations results in hypersensitive cell death that limits pathogen spread (83, 151). In addition, $H_2O_2$ has been shown to act as the primary signal to rapidly and selectively stimulate the transcription of GSTs and glutathione peroxidases (83, 151). The GSTs induced
in turn detoxify lipid peroxides such as 4-hydroxyalkenals or 13-hydroperoxylino-
leic acid by conjugation with GSH (4, 19, 88) and function as glutathione
peroxidases that act on toxic base propenals such as thymidine hydroperoxide
(6). Glutathione peroxidases also catalyze the GSH-dependent reduction/inac-
tivation of H$_2$O$_2$, forming glutathione disulfide (GSSG) and increasing GSH
synthesis by feedback induction (55, 98, 132, 133). Elevated GSH levels induce
transcription of other defense genes, including those for phytoalexin synthesis
that kill the pathogen, pathogenesis-related (PR) proteins such as chitinases that
attack fungal cell walls, and, in some cases, GSTs (31, 83, 95, 165).

Some plants initially infected by pathogens protect themselves against sub-
sequent infection by a process termed systemic acquired resistance (SAR).
Salicylic acid (SA), a phenylpropanoid derivative synthesized during pathogen
attack, is involved in transducing the signal for SAR (14, 70, 117, 158) and has
been proposed to prolong the production of H$_2$O$_2$ by binding and inhibiting
the enzyme catalase, which normally destroys H$_2$O$_2$ (14, 70).

The GST genes known to be induced by pathogen attack include the wheat
GstAI mRNA (35, 95), the potato prp-1 mRNA (150), and the Arabidopsis
AWI24 mRNA (72). In addition, lipid peroxides such as 13-hydroperoxylino-
leic acid and 13-hydroperoxylinolenic acid are known substrates of the
Arabidopsis PM239 GST (4), and cumene hydroperoxide (a model substrate
for glutathione peroxidases) is a substrate of Atpm24 (166). Exogenous GSH
added to cells induces the wheat GstAI and the soybean GH2/4 in ways almost
indistinguishable from those induced by fungal elicitor (31, 83, 95, 165). SA
has been shown to induce Nt103, Nt107, and Nt114 and prp-1 and GmGST26-
A(GH2/4) genes (10, 49, 155, 156), and H$_2$O$_2$ itself induces the soybean
GmGST26-A(GH2/4) gene (83, 155, 156).

H$_2$O$_2$ is thus a key regulatory molecule in the response to infection (83,
151), and its ability to selectively induce a subset of defense genes (cellular
protectant genes like GSTs and glutathione peroxidases) without directly in-
ducing other defense genes indicates that other conditions that generate oxida-
tive stress may activate GST genes through the production of H$_2$O$_2$. Figure 3
shows a model in which conditions that induce oxidative stress also induce
GSTs, with the generation H$_2$O$_2$ and other AOS being a central feature of this
response.

**HEAVY METALS** Heavy metals such as copper and cadmium induce AOS
through a series of redox reactions, leading to oxidative stress and lipid pero-
xidation (27, 28, 50, 69). Heavy metals also induce phytochelatin (PC) synthesis,
heavy metal binding peptides of the structure ($\gamma$-glu-cys)$_{n}$gly that are synthe-
sized directly from glutathione ($\gamma$-glu-cys-gly) (112, 135). Consumption of GSH
by PC synthesis impairs the overall AOS scavenging system, producing a rise
in AOS production (25, 98). Thus, the induction of GST genes by cadmium and
other heavy metals may also be occurring through the generation of oxidative stress. Heavy metals induce the GmGST26-A(GH2/4), parA, NT103, NT107, NT114, and Bz-2 genes (10, 17, 34, 91, 139, 157) and the wheat GST25 and GST26 proteins (95).

OZONE AND ETHYLENE Ozone in plant cells is rapidly dissolved in water and converted to H₂O₂ (126). Ozone exposure also results in stress-induced ethylene biosynthesis (97, 126). Ethylene plays a key role in programmed senescence, which involves membrane degradation and lipid peroxide production as well as GST gene induction (64, 65, 99, 126, 138, 152). The ethylene-regulated carnation GST1 and GST2 genes are induced during programmed senescence, possibly to maintain cell integrity against lipid peroxidation until death, which is necessary for proper nutrient remobilization (65, 99). The ethylene-regulated Arabidopsis GSTpm239 is known to be active against lipid peroxides but is downregulated at anthesis and may thus contribute to senescence by the inability to eliminate the products of lipid peroxidation (4). Ozone induces the Arabidopsis GST1 gene (126).

REGULATION OF GST GENE EXPRESSION

ocs Elements of GST Genes

The mechanism by which xenobiotics and other electrophiles induce animal GST genes has been intensively investigated in animals. Xenobiotic Regulatory Elements (XREs) with the core sequence GCGTG are found in multiple copies in the promoters of P450 genes and GST genes (116). The Antioxidant Response Element (ARE) or Electrophile Responsive Element (EpRE) consists of two nonoverlapping core sequences GTGACA(A/T)(A/T)GC that are
binding sites for the Activator Protein-1 (AP-1) transcription factor complex (18, 43). Daniel has proposed that GST genes containing an EpRE are induced by electrophiles and conditions that generate oxidative stress (18).

Plant GST promoters have not been found to contain functional XREs or EpREs. However, several plant GST promoters were recently found to contain ocs (octopine synthase) elements (167). These 20-bp elements were first identified in promoters of genes from the plant pathogens cauliflower mosaic virus (CaMV) and Agrobacterium tumefaciens and are activated by wounding (167). There is some similarity between ocs elements and EpREs. Both have tandem duplication of binding sites: ocs elements contain a tandem core sequence of ACGT (167), and the EpRE contains tandem AP-1 sites (18, 43); and both elements are binding sites for dimeric b-ZIP transcription factors: Fos/Jun proteins bind the AP-1 site and OCSBF-1 and ASF1 bind the ocs element (18, 43, 167).

The only plant gene promoters containing ocs elements are GSTs: the soybean GmGST26-A(GH2/4); the wheat GstA1; the tobacco Nt103, Nt107, and Nt114; parA and parB; and the Silene GST (155) (Table 2). For at least one of these genes, the soybean GH2/4 gene, the ocs elements have been

**Table 2** Sequence comparisons of ocs-like elements in plant pathogen promoters and plant GST promoters. The ocs-like elements shown are the ocs from the octopine synthase promoter, as-I from the CaMV 25S promoter, nos from the nopaline synthase promoter, GNT25 and GNT1 from the tobacco GST1-1 (NT103) promoter, the wheat Gst1A promoter, the Silene cucubalis GST promoter, the tobacco parA promoter, and the soybean GH2/4, hsp26-A promoter. Core sequences are represented by arrows. Location of the element from the transcription startsite is indicated, as are the number of bases that match the 20-bp ocs consensus element. See text for details.

<table>
<thead>
<tr>
<th>Element</th>
<th>Sequence Comparison</th>
<th>Distance from Transcript Start Site</th>
<th>Matches</th>
</tr>
</thead>
<tbody>
<tr>
<td>ocs</td>
<td>aaACGTAGCGCT+ACGTAC</td>
<td>-175</td>
<td>16/20</td>
</tr>
<tr>
<td>as-I</td>
<td>TGACGTAGCGCT+ACGTAC</td>
<td>-63</td>
<td>16/20</td>
</tr>
<tr>
<td>nos</td>
<td>TGAgcTAAGCGCT+ACGTAC</td>
<td>-111</td>
<td>14/20</td>
</tr>
<tr>
<td>GNT35</td>
<td>T+AgcTAAGCGCT+ACGTAC</td>
<td>-323</td>
<td>14/20</td>
</tr>
<tr>
<td>GNT1</td>
<td>atAgcTAAGCGCT+ACGTAC</td>
<td>-279</td>
<td>13/20</td>
</tr>
<tr>
<td>Wheat GstA1</td>
<td>atccGCTACcaacGcACGTgt</td>
<td>-294</td>
<td>9/20</td>
</tr>
<tr>
<td>Silene GST</td>
<td>caACGTAGCGCT+ACGTAC</td>
<td>-273</td>
<td>12/20</td>
</tr>
<tr>
<td>parA</td>
<td>TACGcAAGCacTGACatCct</td>
<td>-16</td>
<td>13/20</td>
</tr>
<tr>
<td>GH2/4, GmGST26-A</td>
<td>TGAGTAAAGcgCGCTGAGTA</td>
<td>-309</td>
<td>16/20</td>
</tr>
<tr>
<td>Consensus</td>
<td>TGACGTAGCGCTTGAGTA</td>
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shown to confer inducibility by not only strong auxins and SA but also by weak auxins, inactive auxin analogues, and inactive SA analogues, as well as cadmium, GSH, H$_2$O$_2$, methyl jasmonate, and wounding (155, 156).

Thus, the ocs elements within plant GST promoters also appear to be stress-inducible elements that, like AP-1 sites, respond to a variety of electrophilic agents that includes not only biologically active hormones but also inactive hormone analogues and other agents such as heavy metals that generate conditions of oxidative stress (155, 156).

**Other Promoter Elements of GST Genes**

Some GST genes contain promoter elements that are more selectively induced. At least one type I GST, *parB*, does contain two domains also found in Auxin Regulatory Elements (AuxREs) (3, 146). These AuxREs flank an ocs site that responds only to 10–20-fold higher auxin levels, which again suggests that the ocs element mediates a stress response rather than a true auxin response (146). The promoter of the carnation type II GST gene contains a 126-bp ethylene-responsive element (ERE) (64). The ERE contains an 8-bp sequence found in other ethylene-regulated genes and also contains one AP-1 motif (64). The promoter of the potato *prp-1* gene (or *gst1* gene) contains a 273-bp region that is selectively induced by infection with *Phytophthora infestans* and not by other environmental cues (92). The *Bz-2* gene contains numerous promoter sequences necessary for regulation by the maize transcriptional regulators *R* and *C* during anthocyanin biosynthesis (9), and, though not yet tested functionally, *Bz-2* and *GmGST26-A(GH2/4)* (both of which are induced by heavy metals) contain promoters with putative metal regulatory elements (MREs) (17, 91).

**mRNA Stability Determinants in GST Genes?**

Several plant GSTs genes, including *parA*, *parC*, *GmGST26-A(GH2/4)*, *prp-1*, *Bz-2*, and *msrl*, contain one or more copies of the sequence ATTTA in the 3′ untranslated region, a sequence shown to be an instability determinant that targets mRNAs for degradation by RNAses (see 144 and references therein). This instability determinant in the Drosophila *gstD21* gene confers mRNA stability in the presence of pentobarbitol, an inducer of this GST (149). Whether the putative instability determinants in the plant GSTs actually influence mRNA stability, however, has not been tested.

**FATE OF GLUTATHIONE S-CONJUGATES**

**The Glutathione Pump**

In animals, glutathione S-conjugates of xenobiotics as well as endogenous substrates such as leukotrienes are actively eliminated or secreted from the cell
by an ATP-dependent transmembrane glutathione pump or GS-X pump, found in the liver, kidneys, and other organs (63). The pump has at least three essential domains: a P-domain that undergoes phosphorylation, a G-domain that recognizes GSH, and a C-domain with affinity toward the electrophilic moiety of glutathione S-conjugates (62). The GS-X pump is distinct from other ATP-dependent membrane pumps that transport toxic material such as the multidrug resistance protein (MDR), the bile salt ATPase, and the yeast heavy-metal transporter (HMT-1) (62, 63, 93, 108).

In plants, which have no excretion system, soluble glutathione S-conjugates are stored in the vacuole. The glutathione S-conjugates of N-ethylmaleimide and of metolachlor were taken up by vacuoles by an ATPase biochemically identical to the GS-X pump, on the basis of inhibition by vanadate, ATP-dependence, and the recognition of numerous glutathione S-conjugates (93). Anthocyanin-GSH conjugates also appear to be transported into vacuoles by this pump. Thus, anthocyanins are an endogenous substrate for the GS-X pump (90). This common mechanism allows plants to sequester structurally similar but functionally diverse molecules in the vacuole and suggests that a functionally similar mechanism for the transmembrane transport of both xenobiotics and endogenous glutathione S-conjugates operates in both plants and animals.

**Metabolism of Glutathione Conjugates**

In plants, glutathione S-conjugates are metabolized to other products by a complex network of processing reactions (76, 77, 79, 80). The following is a brief summary of known metabolic routes of xenobiotics in plants, as shown in Figure 4. Most herbicide-GSH conjugates in plants are rapidly metabolized by peptidases to yield to cysteine conjugates, as are many glutathione conjugates in animals (76, 77). The cysteine conjugates of herbicides in many plant species are often malonated, or they can undergo transamination to yield a thiolactic acid conjugate (78). In plants, N-malonylcysteine conjugates occupy the equivalent metabolic position in plants as mercuric acids (the acetylated cysteine conjugates that are excreted from the body) occupy in animals (76, 77). N-malonylcysteine conjugates of propachlor, metolachlor, butachlor, EPTC, PCNB, and fluorodifen have been identified from several plants, suggesting that this type of conjugate is formed from many types of glutathione conjugates (78).

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**Figure 4** Metabolism of glutathione conjugates.
It is possible that the fate of anthocyanin-GSH conjugates parallels the fate of herbicide-GSH conjugates, as malonated anthocyanins are commonly found in the vacuole (52, 90). Thus, formation of malonylcysteine derivatives of glutathione S-conjugates might occur for both xenobiotic as well as endogenous compounds. In addition to the formation of soluble conjugates destined for the vacuole, glutathione S-conjugates of both herbicides and of coumarin derivatives such as phytoalexins may become associated with cell wall components such as pectin, hemicellulose, or lignins and targeted for extracellular deposition into the apoplast as an insoluble conjugate termed a “bound residue” (76, 80, 105, 120). This possibility for bidirectional transport of glutathione conjugates differs from metabolism of glutathione S-conjugates in animals, although whether a GS-X pump exists in the plant plasma membrane is unknown (120).

SUMMARY AND PERSPECTIVE

Knowledge about the functions of plant GSTs is growing at a rapid rate. We have known for over 25 years that GSTs are an integral part of the mechanism by which plants defend themselves against herbicide exposure. During the last few years GSTs have been shown to function in the response to hormones and oxidative stress, as well as toward substances such as anthocyanins and IAA, such that we may reasonably conclude that GSTs are involved in numerous processes involving the recognition and transport of electrophilic compounds in plant cells. Although identification of the exact functions of GSTs in many of these processes is still to come, the rapid progress being made in the field of plant GSTs is sure to define these roles more clearly in the near future.

Given the conservation of mechanisms allowing for the recognition and transport of glutathione S-conjugates in both animals and plants, it appears that plants utilize GSTs for as many purposes as animals do. Even within one plant species, GSTs vary greatly in amino acid sequence, regulation, and substrate specificity. It is likely that most, if not all, plants will be shown to have GSTs with interesting substrate specificities and mechanisms of gene regulation. New plant GSTs are being identified and cloned at a rapid rate, and through the use of mutant analysis, genetic engineering, or site-directed mutagenesis, the consequences of manipulating GST levels may provide another way to unravel the functions of GSTs in response to multiple environmental stimuli.

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