Differential Roles of Tau Class Glutathione S-Transferases in Oxidative Stress*

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The plant glutathione S-transferase BI-GST has been identified as a potent inhibitor of Bax lethality in yeast, a phenotype associated with oxidative stress and disruption of mitochondrial functions. Screening of a tomato two-hybrid library for BI-GST interacting proteins identified five homologous Tau class GSTs, which readily form heterodimers between them and BI-GST. All six LeGSTUs were found to be able to protect yeast cells from prooxidant-induced cell death. The efficiency of each LeGSTU was prooxidant-specific, indicating a different role for each LeGSTU in the oxidative stress response mechanism. The prooxidant protective effect of all six proteins was suppressed in the absence of YAP1, a transcription factor that regulates hydroperoxide homeostasis in Saccharomyces cerevisiae, suggesting a role for the LeGSTUs in the context of the YAP1-dependent stress-responsive machinery. The different LeGSTUs exhibited varied substrate specificity and showed activity against oxidative stress by-products, indicating that their prooxidant protective function is likely related to the minimization of oxidative damage. Taken together, these results indicate that Tau class GSTs participate in a broad network of catalytic and regulatory functions involved in the oxidative stress response.

GSTs are soluble proteins composed of two subunits with molecular masses of 22–27 kDa each and until recently have been considered to be mainly homodimeric (for reviews on GSTs see Refs. 1–3). However, a number of reports have established that these proteins can also exist in the heterodimeric form (4, 5). The classic definition of GSTs is based on their ability to catalyze the transfer of the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine (GSH)) to a co-substrate with a reactive electrophilic center, thus forming a polar S-glutathionylated conjugate. This is considered to be a crucial step in the detoxification process, because the produced S-glutathionylated metabolites are either further catabolized and excreted (in animals) or tagged for vacuolar import because of the lack of effective excretion pathways (in plants) (6, 7).

Mechanistically, the binding of GSH to the one active site present per GST subunit results in the ionization of the sulfhydryl group of the tripeptide to yield a highly reactive thiolate anion that is stabilized through hydrogen bonding with an adjacent hydroxyl group. In the mammalian Alpha, Pi, and Mu GSTs, this hydroxyl group is provided by a tyrosine, whereas in most plant enzymes this is provided by a serine (3). This hydrogen-bonding activation results in the lowering of the dissociation constant (pKₐ) of the thiol from 8.7 to 6.0–6.5. GSTs also catalyze GSH-dependent peroxidase reactions by supporting the nucleophilic attack of GSH on hydroperoxides, thus reducing the organic hydroperoxides to the less toxic monohydroxy alcohols. Moreover, GSTs are involved in the isomerization of certain metabolites through the transient formation of a GSH adduct, followed by the spontaneous isomerization of the compound and the release of the isomer and GSH (2). Other cellular roles attributed to GSTs are as follows: the conjugation of natural products (2, 8, 9), the binding and storage of various small molecules (8, 10–12), and the mediation of regulatory signals through protein–protein interactions (13–15).

Recently, a role of GSTs in stress tolerance has been demonstrated. Expression of GSTs in plants is highly responsive to biotic and abiotic stress and to a wide variety of stress-associated chemicals, including 2,4-dichlorophenoxyacetic acid and other synthetic and natural auxins, salicylic acid, methyl jasmonate, abscisic acid, and H₂O₂ (16). Tobacco seedlings overexpressing a tobacco Tau class GST are more tolerant to chilling and osmotic stress than wild-type plants (17), whereas a GST participating in oxidative stress tolerance was discovered in black grass (18). Furthermore, a tomato Tau class enzyme, termed BI-GST, was found to confer resistance to oxidative stress when expressed in yeast cells (19).

BI-GST was isolated by means of a yeast genetic screen intended to identify plant proteins involved in programmed cell death responses (19). Bax, a mammalian proapoptotic protein, was used as the elicitor of cell death in this yeast screen. The lethal phenotype of Bax is characterized by the disruption of the mitochondrial membrane, the dissipation of the mitochondrial membrane potential, and the release of reactive oxygen species into the cytosol. Co-expression of BI-GST in yeast was able to suppress the lethal effect of Bax. To address the function of BI-GST in a plant context, a yeast two-hybrid screen was performed to identify other plant proteins that interact with BI-GST. Most of the isolated interacting clones encoded for genes directly implicated in catalytic or regulatory aspects.
Differential Roles of Tau Class GSTs

The experimental procedures involved the expression of Tau class GSTs (LeGSTUs) in Escherichia coli, which interact by forming heterodimers with BI-GST. BI-GST and the five novel LeGSTUs are found to confer resistance to prooxidant-induced cell death when expressed in yeast. Investigation of the functional and structural characteristics of these enzymes indicates the participation of these enzymes in a complex network of catalytic and regulatory functions involved in oxidative stress response.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen—The pE2G202/LexA-BI-GST construct was generated as described previously (19). To identify interacting proteins, the yeast reporter strain EGY48 (MATa, ura3 trp1 his3 6LexA-operator-LEU2), in which the upstream regulatory elements of the chromosomal LEU2 gene have been replaced by six copies of the LexA operator, was co-transformed with the "bait" pE2G202/LexA-BI-GST plasmid and a tomato cDNA library cloned into the pJG4-5 vector. Library cDNAs are expressed as fusions to a nuclear localization sequence, a portable transcriptional activation domain (the acid blob B42AD), and a hemagglutinin (HA) epitope under the control of the GAL1 promoter (20). Approximately 1 million transformed cells were plated on gal-raff/CDC-his, trp, leu semisolid medium. Isolated library plasmids from a set of 25 growing colonies were reintroduced into the Lexa-BI-GST strains and the EGY48 cells and examined for their ability to grow in the absence of leucine in the medium, to confirm the interaction.

Dimerization of the LeGSTU Heterodimers from Yeast Cell Extracts—The PCR-amplified full-length open reading frame of BI-GST was cloned into the pYES2.1/V5-His6 vector according to the manufacturer’s instructions (Invitrogen). The PCR-amplified full-length open reading frame of BI-GST was cloned into the pCRT7/NT TOPO TA vector, in which the upstream regulatory elements of the chromosomal LEU2 gene have been replaced by six copies of the LexA operator, was inserted into the pCRT7/NT-LEU2 plasmid and a tomato cDNA library cloned into the pJG4-5 vector. Library cDNAs were amplified by PCR and inserted into the pEG202 and pJG4-5 vectors. The C-terminal part of BI-GST, spanning the region from amino acid 103 to the end of the protein, was PCR-amplified by using primers 5'-GCA GAT TCC AGA TTC TAT GAG TAC AGA GAC GAG-3' and 5'-GCA GAT TCC AGA TTC TAT GAG TAC AGA GAC GAG-3' and directly inserted into EcoRI/XhoI pre-digested pEG202 and pJG4-5 vectors. The C-terminal part of BI-GST, spanning the region from amino acid 103 to the end of the protein, was PCR-amplified by using primers 5'-GCA GAT TCC AGA TTC TAT GAG TAC AGA GAC GAG-3' and 5'-GCA GAT TCC AGA TTC TAT GAG TAC AGA GAC GAG-3'. The PCR-amplified full-length open reading frame of BI-GST was PCR-amplified by using primers 5'-GCA GAT TCC AGA TTC TAT GAG TAC AGA GAC GAG-3' and 5'-GCA GAT TCC AGA TTC TAT GAG TAC AGA GAC GAG-3'. The PCR-amplified full-length open reading frame of BI-GST was inserted into the pEG202 and pJG4-5 vectors as above. The ability of the domains to bind to self and to each other was tested by using the two-hybrid assay.

Immunoprecipitation of LeGSTU Heterodimers from Yeast Cell Extracts—The PCR-amplified full-length open reading frame of BI-GST was cloned into the pYES2.1/V5-6His TOPO vector according to the manufacturer’s instructions (Invitrogen). The pYES2.1/BIGST/V5-6His construct expresses BI-GST C-terminally fused to a V5-6His tag. The construct was introduced into EGY48 yeast cells together with each of the pJG4-6/LeGSTU constructs, which express the corresponding GST GST N-terminally fused to a HA tag. 5 ml of overnight cultures growing in glucose-containing media were transferred to 20 ml of gal-raff/CDC-his, trp, leu medium, and allowed to express the proteins for 4 h. At the end of the incubation, the cells were harvested, washed twice with H2O, and resuspended in 1 ml of buffer A (120 mM NaCl, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride) and an equal volume of glass beads. The cells were disrupted by vortexing, and the cellular debris was pelleted by centrifugation. The supernatant was transferred into A-agarose, for 2 h at room temperature. At the end of the incubation, the cells were pelleted, resuspended in fresh medium, and treated with 4 mM H2O2 or 0.1 mM CHP for 3 h. At the end of the incubation, cells were harvested by centrifugation, washed twice with distilled H2O, and re-pelleted. Total glutathione and glutathione disulfide were measured in equal amounts of cellular material according to the method described previously (19). Each determination was performed in triplicate.

Enzyme Assays—1-Chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-(2-methylene-butyryl)phenoxyacetic acid (ethacrynic acid), 1-fluoro-2,4-dinitrobenzene (FDNB), CHP, reduced glutathione, glutathione reductase, and NADPH were obtained from Sigma. 4-Nitrobenzyl chloride (4NBC), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NDB-Cl), trans-2-nonenal, and trans-4-phenyl-3-buten-2-one were obtained from Aldrich. t-BOOH was obtained from Merck. Enzymatic activities with all substrates were determined spectrophotometrically at 340 nm in a Cary spectrophotometer (Hewlett-Packard 8452B). For CDNB, 4NBC, ethacrynic acid, and trans-4-phenyl-3-buten-2-one, the method described by Habig and Jakoby (21) was followed. Assays with NDB-Cl were performed according to Ricci et al. (22), whereas for trans-nonenal the method of Brophy and Barrett (23) was followed. Glutathione peroxidase assays were performed as described previously by a dichloro-leaving group substitution experiments were performed at 1 mM GSH and 0.5 mM CDNB or FDNB. All assays were performed in duplicate at 25 °C.

Kinetic Analysis—Initial reaction rates were determined using the
RESULTS

Screening for Plant Proteins That Interact with BI-GST—To identify plant proteins that interact with BI-GST, we utilized the interaction trap version of the two-hybrid system to screen a tomato library (26). A set of 23 interacting library clones was isolated, and the cDNAs were sequenced. Nine cDNAs were found to represent independent isolates of five proteins homologous to BI-GST. These five novel GSTs, which, like BI-GST, have been considered to be homodimeric enzymes. However, for plant GSTs, there has been evidence for heterodimer formation in proteins purified from sorghum plants or maize proteins expressed in bacteria (4, 5). To confirm the observed interactions, the LexA-BI-GST construct was reintroduced into yeast together with the B42AD fusions of the full-length cDNAs of the five new LeGSTUs. The isolated LeGSTU proteins form heterodimers with each other and with BI-GST. A, EGY48 cells carrying the LexA-BI-GST fusion with B42AD-LeGSTU1 (1), B42AD-LeGSTU2 (2), B42AD-LeGSTU3 (3), B42AD-LeGSTU4 (4), B42AD-LeGSTU5 (5), and B42AD-BI-GST (6) were plated on glu/CM-his, trp, leu (left) and gal-raff/CM-his, trp, leu (right). Expression of the B42AD fusions under the GAL1 promoter enables the cells to grow in the absence of leucine, indicating specific interaction between BI-GST and the five isolated LeGSTUs. B, EGY48 cells expressing the BI-GST (V5—6His) fusion protein and the HA-LeGSTU1, HA-LeGSTU2, HA-LeGSTU3, HA-LeGSTU4, HA-LeGSTU5, and HA-Tvl1 (negative control) proteins, respectively, were precipitated using Ni2+-agarose beads that have affinity for the His6 tag of the BI-GST protein. The precipitating proteins were loaded on two replicate SDS–PAGE gels, blotted, and probed with anti-HA antibody (upper gel) and with anti-V5 antibody (lower gel). BI-GST forms heterodimers in yeast cells with all homologous LeGSTUs. C, bacterially purified proteins (6xHis-Xpress)-BI-GST and LeGSTU5-(V5—6His) were mixed, unfolded with urea, and allowed refold to promote heterodimer formation. LeGSTU5-(V5—6His) was immunoprecipitated using an antibody against the V5 epitope. Western blots were probed with anti-Xpress antibody to detect (6xHis-Xpress)-BI-GST. As a positive control, BI-GST was directly loaded on the gel.

The Isolated LeGSTUs Form Heterodimers between Them and with BI-GST—Glutathione S-transferases have mostly been considered to be homodimeric enzymes. However, for plant GSTs, there has been evidence for heterodimer formation in proteins purified from sorghum plants or maize proteins expressed in bacteria (4, 5). To confirm the observed interactions, the LexA-BI-GST construct was reintroduced into yeast together with the B42AD fusions of the full-length cDNAs of the five new LeGSTUs. As shown in Fig. 1A, BI-GST strongly interacts with all five LeGSTU proteins. The two-hybrid assay was subsequently repeated in the “reverse” orientation. The full-length cDNAs of all five LeGSTU proteins were subcloned into the pEG202 vector, expressed as LexA fusion proteins, and tested against the B42AD fusion of BI-GST. Identical results were observed. Moreover, all five LeGSTUs interacted with each other when tested in all permutations using the interaction mating approach (data not shown).

FIG. 1. The isolated LeGSTU proteins form heterodimers with each other and with BI-GST. A, EGY48 cells carrying the LexA-BI-GST fusion with B42AD-LeGSTU1 (1), B42AD-LeGSTU2 (2), B42AD-LeGSTU3 (3), B42AD-LeGSTU4 (4), B42AD-LeGSTU5 (5), and B42AD-BI-GST (6) were plated on glu/CM-his, trp, leu (left) and gal-raff/CM-his, trp, leu (right). Expression of the B42AD fusions under the GAL1 promoter enables the cells to grow in the absence of leucine, indicating specific interaction between BI-GST and the five isolated LeGSTUs. B, EGY48 cells expressing the BI-GST (V5—6His) fusion protein and the HA-LeGSTU1, HA-LeGSTU2, HA-LeGSTU3, HA-LeGSTU4, HA-LeGSTU5, and HA-Tvl1 (negative control) proteins, respectively, were precipitated using Ni2+-agarose beads that have affinity for the His6 tag of the BI-GST protein. The precipitating proteins were loaded on two replicate SDS–PAGE gels, blotted, and probed with anti-HA antibody (upper gel) and with anti-V5 antibody (lower gel). BI-GST forms heterodimers in yeast cells with all homologous LeGSTUs. C, bacterially purified proteins (6xHis-Xpress)-BI-GST and LeGSTU5-(V5—6His) were mixed, unfolded with urea, and allowed refold to promote heterodimer formation. LeGSTU5-(V5—6His) was immunoprecipitated using an antibody against the V5 epitope. Western blots were probed with anti-Xpress antibody to detect (6xHis-Xpress)-BI-GST. As a positive control, BI-GST was directly loaded on the gel.
To verify further the interactions detected, we assessed the direct binding of BI-GST to the LeGSTUs in yeast cells by co-precipitation. The full-length cDNA of BI-GST was expressed in yeast cells as a fusion to the V5 antigenic tag and a His$_6$ tag. EGY48 cells co-expressing BI-GST-(V5–6xHis) and the different LeGSTU molecules as HA fusions were lysed in mild detergent buffer, and His-tagged BI-GST was precipitated using Ni$^{2+}$-agarose beads, resolved by SDS-PAGE, and blotted. The presence of BI-GST was detected in replicate blots using an antibody against the V5 epitope. Co-precipitating LeGSTUs were detected using an antibody against the HA epitope. Binding of the LeGSTU proteins to BI-GST is specific, because the unrelated mammalian ankyrin protein Tvl-1 is not precipitated (Fig. 1B).

To assess whether recombinant purified proteins form heterodimers in vitro, bacterially purified BI-GST fused in the N terminus to an Xpress epitope-His$_6$ tag and LeGSTU5 fused in the C terminus to a V5 epitope-His$_6$ tag were mixed, denatured in urea, and allowed to refold. LeGSTU5 was immunoprecipitated by using an anti-V5 antibody, and the presence of BI-GST was detected with an antibody against the Xpress epitope. BI-GST co-precipitated specifically with LeGSTU5 (Fig. 1C). To confirm that the interaction of BI-GST with the five LeGSTUs is due to heterodimerization, the dimerization domain of BI-GST (residues 31–106, as judged by the structure of a wheat Tau class enzyme by Thom et al. (28), and the molecular model described below) was assessed for interaction with all five LeGSTUs by the yeast two-hybrid system. The dimerization domain of BI-GST was capable of binding all other LeGSTUs, although the strength of the interaction was weaker than that observed between two full-length proteins (data not shown).

Expression of the LeGSTU Proteins in Yeast Enhances Survival Under Oxidative Stress—The model eukaryote Saccharomyces cerevisiae is ideal for investigating the properties of antioxidant enzymes, because it is a genetically well defined system and its defense responses against reactive oxygen species are well characterized (29, 30). Screens for plant proteins conferring increased resistance to oxidative stress have identified several antioxidant proteins (31–33). It has been shown previously (19) that expression of BI-GST in yeast had a protective effect. The expression levels, because cell extracts of yeast cells expressing the various LeGSTUs all showed similar protein levels in Western blot analysis (data not shown). The specificity in the protection conferred by the different LeGSTUs against the three prooxidants examined indicates a variation in the mechanism by which the different prooxidants exert their lethal action and/or in the substrate specificity of each enzyme.

The Prooxidant-protective Function of LeGSTUs Requires the YAP1-responsive Mechanism—YAP1 regulates the transcription of a large array of genes involved in the oxidative stress response (29, 34–41). To assess whether the anti-oxidant protection conferred by the LeGSTUs involves a YAP1-dependent function, the prooxidant assays were repeated on a ΔYAP1 genetic background. Any degree of protection conferred by the LeGSTUs against the three prooxidants examined indicates a variation in the mechanism by which the different prooxidants exert their lethal action and/or in the substrate specificity of each enzyme.

**Fig. 2.** The LeGSTUs exhibit differential effects against prooxidants, and their action is dependent on the presence of YAP1. Freshly grown wild-type EGY48 or ΔYAP1 cells expressing BI-GST and the newly isolated LeGSTUs were treated with restrictive concentrations of three prooxidants (4.5 mM $\text{H}_2\text{O}_2$, 1.25 mM t-BOOH, and 0.1 mM CHP) for 6 h and plated on rich media. Cells were enumerated before and after treatment, and the percentage of surviving cells was determined. EGY48 indicates wild-type or ΔYAP1 cells that do not contain any plasmid, whereas pYES indicates wild-type or ΔYAP1 cells containing empty pYES vector and are used as controls. Results represent the average of three independent determinations.
ΔYAP1 strain could be due to the inability of these cells to maintain physiological levels of reduced glutathione, thus significantly reducing the catalytic activity of the LeGSTUs. To test this hypothesis, we determined the intracellular levels of oxidized and reduced glutathione in prooxidant-challenged cells. When cells were treated with H2O2, total glutathione levels remained constant both in wild-type and ΔYAP1 cells (Fig. 3). Examination of GSSG levels revealed that there was no significant increase of oxidized glutathione after treatment with H2O2 in either strain. However, there was a significant increase in GSSG when yeast cells expressing BI-GST were challenged. This resulted in a 25–30% decrease of the reduced form in both strains. The inability of the LeGSTUs to rescue ΔYAP1 cells is not likely due to glutathione depletion, because the drop in GSH levels was not sufficient in abolishing enzyme activity, and it was observed in both wild-type and ΔYAP1 cells. It appears that the observed decrease was due to the presence of BI-GST, because no increase in GSSG levels was observed in cells lacking BI-GST.

In contrast, when yeast cells were challenged with CHP, there was a significant decrease in GSH levels in the ΔYAP1 strain but an important increase in wild-type cells (Fig. 3). Expression of BI-GST in ΔYAP1 cells restored GSH to ~70% of normal levels. Moreover, treatment of wild-type cells with CHP resulted in an increase in total glutathione both in the presence and absence of BI-GST (Fig. 3). It appears that the effect on the glutathione levels was prooxidant-specific and further supports the notion that the mechanism by which each compound causes cell death is different.

Additive and Cooperative Effects of LeGSTU Coexpression in Oxidative Damage—The ability of the six proteins to cooperate functionally in the protection against prooxidants was examined by expressing BI-GST together with each different LeGSTU in yeast cells challenged with prooxidants. In the case of H2O2- or t-BOOH-treated cells, only an additive effect in the viability of cells expressing LeGSTU pairs was evident (Fig. 4). However, CHP-treated cells co-expressing BI-GST with either LeGSTU1, LeGSTU2, or LeGSTU4 exhibited substantially higher degrees of viability in comparison to cells expressing a double dosage of BI-GST (BI-GST expressed from two plasmids) or BI-GST together with the unrelated protein Tvl-1 (Fig. 4). The three enzymes, LeGSTU1, LeGSTU2, and LeGSTU4, afforded the least amount of protection against CHP when expressed alone in yeast (Fig. 2). By contrast, LeGSTU3 and LeGSTU5, which conferred resistance against CHP similar to that observed with BI-GST when expressed alone, failed to yield viability levels higher than the double dosage of BI-GST when co-expressed with the latter (Fig. 4). This cooperativity in the mode of LeGSTU1, LeGSTU2, and LeGSTU4 action may be attributed to a functionality complementary to that of BI-GST.
or a modification of the antioxidant capacity of BI-GST due to heterodimerization.

Protein Expression and Purification—The biochemical properties of the six LeGSTU proteins were studied in vitro in order to identify functional characteristics related to their ability to protect cells from prooxidant-induced cell death. To this end, and in order to facilitate detection and purification, BI-GST and the five other Tau class GSTs were expressed in bacteria as fusions to an N-terminal 6-histidine tag and an Xpress antigenic epitope (Invitrogen). Expression in E. coli (BL21) cells, followed by Ni²⁺-affinity purification, resulted in protein preparations with higher than 90% homogeneity for all proteins, as judged by SDS-PAGE. With BI-GST, the Ni²⁺-affinity purification step was followed by purification on a GSH-affinity column. This resulted in an almost homogenous preparation, but offered only a modest increase (less than 10%) in the specific activity of CDNB. This was taken as an indication that almost all the protein was folded correctly and able to bind GSH. Purification of the other LeGSTUs on a GSH-affinity column resulted in significant loss of protein in this step, suggesting a lower affinity of these proteins for GSH. This behavior was not due to a high proportion of inactive protein in the preparation because no significant difference in the specific activity was observed prior to and after the affinity purification step.

Substrate Specificity—GSTs catalyze a broad range of reactions, with different members of the family exhibiting quite varied substrate specificity. The substrate specificity of the isolated LeGSTUs was investigated in order to identify catalytic activities that may be related to their biological function. To this end, a broad range of substrates was examined, including CDNB, NBD-Cl, 4NBC, CHP, t-BOOH, hydrogen peroxide, 4-hydroxy-nonenal, trans-trans-nona-2,4-dienal, ethacrynic acid, and trans-4-phenylbut-3-en-2-one. CDNB is the classical GST substrate. It is used widely in the detection and determination of GST activity, despite the fact that a number of GSTs have very poor activity with this substrate. NBD-Cl is another substrate for the GSH conjugation reaction of GSTs, exhibiting high activity with the mammalian Alpha class GSTs (22). The rest of the substrates examined are related to the role of GSTs in the detoxification of oxidative stress by-products. The reactive oxygen species attack on cell components results in the production of organic hydroperoxides and activated alkenes, epoxides, and quinones. GSTs participate in oxidative stress defense mechanisms by catalyzing GSH-dependent reactions that inactivate such products by conjugation or reduction. Organic hydroperoxides, produced by free radical attack on membrane lipids, are reduced by the glutathione peroxidase activity of GSTs (2). Cumene hydroperoxide and t-butyl hydroperoxide have extensively been used as model substrates for the determination of such an activity. Oxidative stress also results in the production of cytotoxic alkenals. One such example is 4-hydroxy-nonenal, which was found to be generated by peroxidation of arachidonic acid in rat microsomes exposed to oxidative stress (42). In plants, at least one Phi class GST from sorghum has been found to be active in the GSH conjugation-mediated detoxification of 4-hydroxy-nonenal (5). Ethacrynic acid is a pheny lacetic acid derivative that contains an electrophilic group, similar to the α-β-alkenals, and has also been widely studied as a GST substrate.

| Substrate (concentration) | Enzyme specific activity | | | | |
|--------------------------|--------------------------|-----------------|-----------------|-----------------|-----------------|
|                          | [GSH] BI-GST U1-1 U2-2 U3-3 U4-4 U5-5 | μmol·mg⁻¹·min⁻¹ | μmol·mg⁻¹·min⁻¹ | μmol·mg⁻¹·min⁻¹ | μmol·mg⁻¹·min⁻¹ | μmol·mg⁻¹·min⁻¹ |
| CDNB (1 mM) | 2 mm | 9.22 ± 0.91 | 0.07 ± 0.01 | 2.01 ± 0.20 | 5.55 ± 0.61 | 0.16 ± 0.02 | 0.06 ± 0.01 |
| NBD-Cl (0.2 mm) | 0.5 mm | 12.38 ± 1.61 | 0.15 ± 0.03 | 1.25 ± 0.14 | 5.19 ± 0.63 | 0.09 ± 0.02 | 0.30 ± 0.04 |
| 4-NBC (0.5 mm) | 5 mm | 1.35 ± 0.17 | 0.19 ± 0.03 | 0.09 ± 0.02 | 0.01 ± 0.01 | 0.08 ± 0.01 | 0.42 ± 0.05 |
| Cumene hydroperoxide (0.05 mg/ml) | 2 mm | 0.21 ± 0.03 | 0.18 ± 0.03 | 0.19 ± 0.03 | 0.06 ± 0.01 | 0.17 ± 0.03 | 0.02 ± 0.01 |
| t-BOOH (1.5 mM) | 2 mm | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.02 ± 0.1 | ND | 0.06 ± 0.1 | ND |
| H₂O₂ (1 mM) | 2 mm | 0.01 ND | 0.02 ND | 0.01 ND | 0.05 ± 0.01 | ND |
| Ethacrynic acid (0.2 mM) | 0.25 mm | 0.17 ± 0.03 | 0.06 ± 0.01 | ND | 0.13 ± 0.03 | 0.15 ± 0.03 | 0.26 ± 0.04 |
| trans-4-Phenylbut-3-en-2-one (0.0125 mM) | 0.25 mm | 0.06 ± 0.01 | ND | 0.03 ± 0.01 | 0.06 ± 0.01 | 0.08 ± 0.01 | ND |
| trans-trans-Nona-2,4-dienal (0.05 mM) | 2.5 mm | 0.09 ± 0.01 | ND | 0.02 ± 0.01 | 0.08 ± 0.01 | 0.04 ± 0.01 | ND |
| 4-Hydroxynona-2-enoal (0.1 mM) | 0.5 mm | ND | ND | ND | ND | ND |
| trans-Nona-2-enoal (0.025 mM) | 1 mm | ND | ND | ND | ND | ND |

¹ ND, non-detectable (lower than 0.01 μmol·mg⁻¹·min⁻¹).
reduction to the other serine-containing LeGSTUs. This suggests that either serine-mediated stabilization of the thiolate ion is not required in the glutathione peroxidase mechanism of GSTs or that another residue may play the role of serine in LeGSTU1-1.

LeGSTU5-5, BI-GST, LeGST3-3, and LeGSTU4-4 showed activity with ethacrynic acid (Table I). This was comparable with the activity exhibited by other plant and mammalian GSTs (A1-A1 and B1-B1 from sorghum, ZmGSTI, ZmGSTV, ZmGST19, and ZmGST4 from maize and GSTA1-1, GSTA2-2, and GSTA3-3 from human) and only lower to human GSTA4-4, an enzyme particularly potent with activated-alkene substrates (5, 48–50). No enzyme was active with trans-2-nonenal or 4-hydroxynonenal, but BI-GST, LeGSTU2-2, LeGSTU3-3, and LeGSTU4-4 showed catalytic activity in additional reactions with trans-trans-nona-2,4-dienal and trans-4-phenylbut-3-en-2-one (Table I). Human and rat GSTT2-2 exhibited rates of GSH addition to trans-trans-nona-2,4-dienal similar to the ones observed with the LeGSTUs (51).

Overall, the CDNB and NBD-Cl conjugation activities of some members of the group (namely BI-GST, LeGSTU2-2, and LeGSTU3-3) indicate a possible role for these enzymes in the detoxification of exogenous compounds. However, the activities of the six LeGSTUs against H2O2 or the organic hydroperoxides CHP and t-BOOH do not correspond to their efficiency in protecting yeast cells against these prooxidants. In the case of CHP, for example, LeGSTU5-5 was the most efficient enzyme in protecting yeast cells but the one with the lowest in vitro specific activity against this compound. Also, LeGSTU4-4, which was the only enzyme showing detectable levels of activity with H2O2 in vitro, was the least efficient in protecting from H2O2 insults in vivo. These results indicate that the mode by which the LeGSTUs protect yeast cells from prooxidant oxidative damage is probably not by direct catalytic removal of the prooxidants. The antioxidant effect of LeGSTUs may be related to their ability to remove harmful oxidative stress by-products, because some members of the group show activity against model substrates related to oxidative stress (ethacrynic acid, trans-trans-nona-2,4-dienal, and trans-4-phenylbut-3-en-2-one).

Kinetics of the Conjugation Reaction—To study the catalytic properties of these enzymes and to compare their mechanism to members of the other classes, the three most potent enzymes with CDNB and NBD-Cl were selected for a detailed kinetic study. Initial velocity data for the BI-GST, LeGSTU2-2, and LeGSTU3-3-catalyzed reactions with CDNB and NBD-Cl were collected both by varying the compound concentration at fixed concentrations of GSH and by varying the concentration of GSH at constant concentrations of the compounds (Fig. 5). BI-GST exhibited hyperbolic kinetics under all conditions examined (Fig. 5, A and B). A number of GSTs have been reported to follow a rapid equilibrium random sequential Bi-Bi mechanism. Least squares regression analysis revealed that the rapid equilibrium random sequential model was the one that best fit the BI-GST data. Global fitting of the experimental data revealed that there was no significant improvement to the fit when the coupling factor \( \alpha \) was allowed to fluctuate instead of being fixed at a constant value of 1 (i.e. there was no significant difference in the sum of squares between the two fits to justify the use of one extra parameter). For this, the equilibrium binding constants for CDNB and GSH were determined by global fitting to the rapid equilibrium random sequential model with the value of the coupling factor fixed at 1. BI-GST catalyzes the conjugation of GSH to CDNB with a \( k_{\text{cat}} \) of 5.6 s\(^{-1}\), whereas the equilibrium dissociation constant for GSH is 457 \( \mu \)M, well in the range of \( K_{\text{GSH}} \) values reported for other GSTs (0.2–0.6 mM). The kinetic parameters for the conjugation of CDNB by BI-GST are summarized in Table II. With NBD-Cl, the experimental data conformed well to the rapid equilibrium random model with a coupling factor \( \alpha = 0.21 \) indicating positive synergism between GSH and NBD-Cl binding (Fig. 5, C and D). The rate constant \( k_{\text{cat}} \) was 4.4 s\(^{-1}\), whereas \( K_{\text{GSH}} \) and \( K_{\text{NBD-Cl}} \) were 361.3 and 63.8 \( \mu \)M, respectively (Table II).

In the case of LeGSTU2-2, again no significant synergism between GSH and CDNB binding was observed. However, the affinity of LeGSTU2-2 for both GSH and CDNB is significantly lower than that of BI-GST. \( K_{\text{GSH}} \) and \( K_{\text{CDNB}} \) values were determined to be 1503 and 2051 \( \mu \)M, respectively (Fig. 5, E and F, and Table II), explaining the poor retention of this enzyme to the GSH-affinity matrix. In the case of NBD-Cl, there is positive synergism between the G and H sites of LeGSTU2-2, not

![Fig. 5. Kinetic analysis of BI-GST and LeGSTU2-2 with CDNB and NBD-Cl.](http://www.jbc.org/doi/10.1074/jbc.M110.144197)
observed with CDNB (Fig. 5, G and H, and Table II). GSH binding increases the affinity for NBD-Cl by a factor of 2 (α = 0.46). K_{NBD-Cl} was higher than the one for BI-GST at 185 μM.

LeGSTU3-3 Exhibits Substrate-dependent Cooperativity—LeGSTU3-3 exhibits a particular kinetic behavior with both substrates. With NBD-Cl, hyperbolic kinetics, characterized by strong positive synergism between the G and H sites (α = 0.009), are observed (Table III). The rapid equilibrium random sequential Bi-Bi model again best describes the experimental data. LeGSTU3-3 has poor affinity for both GSH (K_{GSH} = 1911 μM) and NBD-Cl (K_{NBD-Cl} = 16 μM) but an efficient catalytic rate constant (k_{cat} = 26.3 s⁻¹). With CDNB, the kinetics are non-hyperbolic (Fig. 6A). The deviation from the hyperbolic behavior is observed only when CDNB is the varied substrate but not when GSH is varied at fixed CDNB concentrations (Fig. 6B). This behavior can be explained by either an extreme case of a steady-state random Bi-Bi mechanism (52) or by a positive homotropic behavior of CDNB. To distinguish between these two possibilities, an activation experiment can be performed (53). 4NBC is a molecule closely related structurally to CDNB, but one with which LeGSTU3 exhibits limited reactivity (Table I). As such, 4NBC can be considered as a competitive inhibitor of the CDNB conjugation reaction. The initial rate of CDNB conjugation was determined in the presence of increasing concentrations of 4NBC. At both combinations of CDNB and GSH concentrations employed (0.2 mM CDNB and 0.1 mM GSH or 0.2 mM CDNB and 1 mM GSH), addition of 4NBC resulted in almost the doubling of the CDNB conjugation rate (Fig. 6C). In the case of a steady-state random sequential mechanism, increasing concentrations of 4NBC would inhibit the reaction. Hence, the activation effect of 4NBC can only be explained in the context of a positive homotropic behavior of LeGSTU3-3 with CDNB.

Bound GSH Has Relatively High pK_a—A crucial feature of the catalytic mechanism of GSTs is the stabilization of the thiolate ion of the bound glutathione. This results in the lowering of the pK_a value of the glutathione sulhydryl group by 2.5–3 pH units. The pH dependence of the CDNB conjugation reaction of the three LeGSTUs was studied in order to investigate the similarity of the isolated Tau class enzymes with enzymes from other classes in this aspect of the catalytic mechanism. The specificity constant (k_{cat}/K_m or k_{cat}/S_0.5) in the case of LeGSTU3-3) was determined in the pH range from 5.0 to 8.5 and plotted against pH. The pK_a was determined by fitting the data to Equation 2 under “Experimental Procedures.” The glutathione sulhydryl in BI-GST was found to have an unusually high pK_a of 7.34 ± 0.11 (Fig. 7A and Table III), compared with 6.0–6.6 in other GSTs. Examination of LeGSTU2-2 and LeGSTU3-3 also revealed relatively high pK_a values of 6.88 ± 0.08 and 7.01 ± 0.13, respectively (Fig. 7B and Table III).

Determination of the Rate-limiting Step—FDNB was used in a leaving group substitution experiment in order to determine the rate-limiting step in the conjugation reaction of the three LeGSTUs with CDNB. The presence of fluorine in FDNB (as opposed to chlorine in CDNB) provides a more electrongative leaving group, thus resulting in a higher rate of GSH conjugation. In the case of the non-enzymatic conjugation of FDNB to GSH, the reaction rate was 30–35-fold higher than the respective CDNB conjugation rate (Table IV). When the BI-GST-catalyzed rate of FDNB conjugation was measured, this was similar to the one determined for CDNB, suggesting that the chemical step is not the rate-limiting step in the mechanism of this enzyme (Table IV). As has been proposed for other members of the GST family, this is likely to be a much slower subsequent event related to a protein conformational change or product release (54, 55). In the case of LeGSTU2-2, the rate of the FDNB reaction is almost 30-fold higher than the corresponding CDNB rate, clearly suggesting that the rate-limiting step in this reaction is a chemical event (Table IV).

Molecular Modeling—A large number of GST structures is available, representing enzymes from all GST classes. Despite the low sequence homology between classes (less than 30%), the overall polypeptide fold is very similar among the crystal structures so far obtained (1, 3). With respect to the plant Tau class GSTs, the first crystal structure has been published recently by Thom et al. (28) who reported the structure of a Tau class protein from wheat. This enzyme, TaGSTU4-4, shares sequence similarity ranging from 32 to 43% with the six tomato GSTs reported in this paper. The consistency in the fold of GSTs and the availability of the structure of a Tau class protein prompted us to investigate the possibility of constructing molecular models for our six proteins. We employed the web-based program Swiss-Model for the initial construction of the models and the accompanying program DeepView for the refinement of the structures (details of the modeling procedure are reported under “Experimental Procedures”). Fig. 8 depicts the amino acid alignment used in the modeling process. All six models display the classical GST fold and have root mean square values to TaGSTU4-4 ranging from 0.61 to 1.37 Å (Fig. 9 and Table V). However, they show distinct differences in helices α4 and α5 (numbering of helices is consistent with the other GSTs) in the surface buried area in the dimer interface and in the overall surface charge. LeGSTU4-4 contains a short α4 helix followed by a rather extended loop that connects it to helix α5 (Fig. 9). This is in contrast to BI-GST, LeGSTU1-1, LeGSTU2-2, and LeGSTU3-3, which possess a longer α4 helix.
and a significantly shorter connecting loop, as is also the case for TaGSTU4-4. In the LeGSTU5-5 model both helices α4 and α5 are interrupted by small loops (Fig. 7). This was also observed in helix α4 of TaGSTU4-4 (28). The dimer-buried surface area in the BI-GST model is significantly smaller than that of the other five LeGSTU models. BI-GST has a total buried surface of 1153 Å² as opposed to 1844–2136 Å² of the other proteins (Table V). This difference is mainly due to the composition of amino acids in the variable region at the end of helix α4 which, in the case of the larger dimer interface proteins, consists of side chains that protrude in the region between the two subunits, thus increasing the total area of contact between the two monomers. This is clearly evident in the comparison between BI-GST and LeGSTU4-4 surface representations shown in Fig. 9 (bottom panel). The position of the loop linking helices α4 and α5 of LeGSTU4-4, which now occupies the region between the two monomers of LeGSTU4-4, is another factor contributing to the size of the dimerization interface. A smaller dimer interface in the case of BI-GST may be an important factor in heterodimer formation or interaction with other non-GST proteins.

**DISCUSSION**

Research on plant GSTs has primarily focused over the years on the capacity of the molecules to detoxify xenobiotics through their ability to conjugate GSH to electrophilic substrates, such as certain types of herbicides, and render them more water-soluble and less toxic. The current study focuses on the ability of these enzymes to protect yeast cells from oxidative damage. All six LeGSTUs studied appear to play a role in the defense against oxidative stress. BI-GST and LeGSTU3-3 not only suppress the lethal phenotype of Bax (19) but also rescue cells from prooxidant-induced cell damage. LeGSTU5-5 confers resistance to organic hydroperoxides (t-BOOH and CHP), whereas LeGSTU2-2 protects cells from H2O2 and t-BOOH.

| pKₐ values of the bound GSH | BI-GST | LeGSTU2-2 | LeGSTU3-3 |
|-----------------------------|--------|-----------|-----------|
| pKₐ | 7.34 ± 0.11 | 6.88 ± 0.08 | 7.01 ± 0.13 |

| Leaching group substitution | Ratio of FDNB/CDNB spontaneous reaction rates | Ratio of FDNB/CDNB enzyme-catalyzed reaction rates |
|-----------------------------|-----------------------------------------------|--------------------------------------------------|
| BI-GST                      | 32.56 ± 3.37                                 | 0.93 ± 0.12                                      |
| LeGSTU2-2                   | 29.37 ± 3.11                                 | 27.85 ± 3.76                                     |
| LeGSTU3-3                   | 35.32 ± 4.41                                 | 6.76 ± 1.54                                      |

* I. Dimitrova, unpublished results.
but not from H₂O₂ or CHP. The prooxidant-specific action of LeGSTUs may be attributed to a variability in the mechanism by which the different prooxidants exert their lethal action and in the mechanisms developed by cells to avert oxidative damage. This is further supported by the observed prooxidant-dependent variation in the intracellular levels of oxidized and reduced glutathione. Moreover, dissection of the \textit{S. cerevisiae} oxidative stress-response pathways suggested the existence of overlapping but distinct defense systems dealing with the products of peroxidation. Pretreatment of yeast cells with sub-lethal doses of linoleic acid hydroperoxide conferred resistance to H₂O₂ but not to the organic hydroperoxides tert-BOOH and CHP (56). In the case of the antioxidant behavior of the LeGSTU molecules, these results suggest a specialization inside plant cells to cope with reactive oxygen species and environmental injury.

The protection conferred by the LeGSTUs disappeared on a \textit{YAP1} genetic background, indicating that the anti-oxidant effects of LeGSTUs are dependent on the \textit{YAP1}-responsive machinery. The \textit{YAP1} transcription factor, a redox homeostasis regulator in yeast, controls the transcription of a large array of genes involved in the oxidative stress response (29, 34 – 41).

Two of the genes regulated by \textit{YAP1} are \textit{GSH1} and \textit{GLR1}, encoding for \textit{γ}-glutamylcysteine synthase (the first enzyme in glutathione biosynthesis) and glutathione reductase (34 – 36). This points to a mechanism where the inability of \textit{YAP1} cells to maintain the depleted GSH pool under conditions of oxidative stress results in the inactivation of the LeGSTUs. However, determination of the levels of oxidized and reduced glutathione in wild-type and \textit{YAP1} cells shows that, in the absence of \textit{YAP1}, glutathione levels in challenged cells are sufficient for BI-GST to carry out its catalytic function. Thus, another \textit{YAP1}-regulated functionality is responsible for the inability of BI-GST and the other LeGSTUs to rescue \textit{YAP1} cells.

Recent work by Collinson and co-workers (57) showed that overexpression of two yeast glutaredoxins in \textit{S. cerevisiae} enhanced resistance of cells to hydroperoxides, including hydrogen peroxide, tert-butyl hydroperoxide, and CHP. Most interesting, the mechanism of resistance is dependent on GSH conjugation and conjugate removal from the cells, because protection is abolished in strains lacking glutathione S-transferases (GTT1 and GTT2) or the GS-X pump (YCF1). Thus, the
participation of the LeGSTUs in enhancing protection against prooxidants may not primarily involve their peroxidase capacity but also additional interactions with the stress-responsive machinery of the cell, resulting in the removal of toxic by-products resulting from the prooxidant treatments. Such a role for the LeGSTUs in the GSH conjugation-mediated detoxification of harmful by-products is consistent with the dependence of their action on the presence of YAP1, because this transcription factor also regulates several genes that could be involved in the first steps of such a mechanism, including the TSA1 and AHP1-encoded thioredoxin peroxidases (41), thioredoxin 2 (38), thioredoxin reductase-1 (39, 40), and glutathione peroxidase (37). Enzymes regulated by YAP1 mainly function at a first stage of the stress response by removing dangerous reactive oxygen species, whereas the LeGSTUs may function primarily at a second stage, at which oxidative stress by-products are removed. Thus, failure to up-regulate enzymes of the first stage in ΔYAP1 cells renders them unable to cope with prooxidants despite the presence of the LeGSTUs.

The six proteins show important differences in substrate utilization, kinetic properties, and structural features. BI-GST, LeGSTU2-2, and LeGSTU3-3 exhibit GSH conjugation activity comparable with other plant GSTs, suggesting that they may also function in the detoxification of exogenous compounds. Members of the group showed in vitro activity against compounds related to the oxidative metabolism (e.g. ethacrynic acid) supporting the role for these enzymes in the removal of oxidative stress by-products. Moreover, the prooxidant-dependent protection pattern did not correspond to the in vitro specific activity of these enzymes toward the respective prooxidants, further suggesting that the action of the enzymes is not directly related to the removal of the prooxidant but rather focuses on the minimization of oxidative damage.

There are marked differences in substrate specificity between the enzymes studied. The ability of these six proteins to heterodimerize is another factor that may contribute significantly to the variability in substrate specificity. Although no alterations in the kinetic properties of the individual subunits of the heterodimer were reported for Tau class heterodimers from sorghum or maize (4, 5), it is possible that heterodimers exhibit altered specificity toward their physiological substrates in planta. Considering the fact that Tau class GSTs have been extensively duplicated in the course of evolution, and that plants examined to date consist of an average of 20–25 such genes, the capacity to heterodimerize together with the possibility of altered substrate specificity in the heterodimer would generate a vast diversity of catalytic specificities enabling plants to cope with their adverse environment.

We studied the ability of these enzymes to co-operate in vitro by testing the ability of the other five LeGSTUs to enhance the protection against prooxidants conferred to yeast cells by BI-GST. LeGSTU1 and LeGSTU4, two members of the group that appeared unable to efficiently protect yeast cells from Bax- or prooxidant-induced cell death, had a profound effect on cell viability when expressed together with BI-GST. Although the possibility that these two proteins act independently but complementary to BI-GST cannot be ruled out, it seems more likely that formation of the BI-GST/LeGSTU1 or BI-GST/LeGSTU4 heterodimers (confirmed by co-precipitation, Fig. 1B) is providing a functionality important for the removal of specific cytotoxic by-products. This is supported by the marked inability of LeGSTU1 and LeGSTU4 to avert prooxidant-induced cell death when expressed alone in the yeast system and the observation that the other, more potent, LeGSTUs offered an additive rather than a cooperative effect against all prooxidants tested when co-expressed with BI-GST.

The GSH conjugation reaction of three of the LeGSTUs was studied in detail. All three enzymes appear to follow rapid equilibrium random sequential Bi-Bi kinetics. In BI-GST, the rate-limiting step in the reaction with CDNB is product release. However, with LeGSTU2-2 and LeGSTU3-3, a chemical event appears to be rate-limiting. One of the GSTs studied, LeGSTU3-3, exhibited substrate-dependent cooperativity. For most GSTs it has been proposed that they follow a non-homotropic behavior. Because the endogenous substrates of GSTs are not sufficiently known, this finding suggests that these enzymes may indeed possess homotropic characteristics toward their in vivo substrates.

For BI-GST, the GSH equilibrium dissociation constant (K_{GSH}) is well in the range of values observed in other GSTs. However, both LeGSTU2-2 and LeGSTU3-3 have K_{GSH} values above 1.5 mm. This can explain the observation that only BI-GST could be retained efficiently on a GSH-affinity matrix. An important aspect of the mechanism of the conjugation reaction is the stabilization of the thiolate ion of GSH by the active site Ser, which results in the lowering of the pH of the bound glutathione to 6.0–6.5. Bound-GSH pH values in the three enzymes were well above the average for other GSTs, ranging from 6.89 (for LeGSTU2-2) to 7.34 (for BI-GST). Taken together, these observations suggest that these enzymes may not be optimized solely for a catalytic function but that they may also be performing additional roles inside the cell, such as the participation in regulatory networks. From the structural viewpoint the variability observed in helices a4 and a5 and the connecting loop(s) might be important for the interaction with other proteins and/or ligand-induced conformational changes. Indeed, in the two-hybrid screen, except for the nine clones coding for the five LeGSTUs, the isolated clones mainly represented proteins that have been directly implicated in resistance to abiotic environmental stress. A recently identified novel facet of GST function involves the interactions with other proteins and the regulatory effects mediated through them. In mammals, Adler et al. (13) presented evidence for direct interaction between GSTP and c-Jun N-terminal kinase, in which GSTP binds as a monomer and inhibits c-Jun N-terminal kinase phosphorylation and activity, suppressing reactive oxygen species-mediated signaling. In the case of mGSTM1-1, it was shown that the protein interacts with the N-terminal portion of the apoptosis signal-regulating kinase (ASK1), suppressing its activity in a way that is independent of its transferase activity (15). Furthermore, the Fanconi anemia group C protein was shown to bind directly to GSTP1, preventing the formation of disulfide bonds that would inactivate the latter, forcing the cells to undergo apoptosis (14). With regard to plant GSTs, this is the first evidence of protein-protein interactions with non-GST proteins.

The large repertoire of GST heterodimers that is transcriptionally controlled to respond to different environmentally adverse situations, the selective capacity to detoxify reactive oxygen species, protect organelles, and prevent cellular damage, as well as the potential modulating activity mediated through protein interactions on the stress-responsive cascades would provide a flexibility necessary for a sessile organism to cope with changing environmental conditions. Most interesting, GSTs are frequently significantly more abundant in crops than in the competing weeds (almost 20-fold). Enhanced GST expression, at least for wheat and its relatives, is a characteristic of the progenitor Triticum species, selected in the domestica-
Differential Roles of Tau Class GSTs

Enhance resistance to chilling and salt stress in seedlings (17). Selective overexpression of pairs of Tau class GSTs with complementary functionalities may thus offer an attractive way to incrementally enhance the capacity of the plant to cope with stress, avoiding undesirable consequences to plant growth due to persistent activation of the stress-response machinery.

Future work on elucidating the mechanism of LeGSTU protection against prooxidants and Bax-induced lethality will focus on screening deletion mutant yeast strains to identify genes that cooperate with the LeGSTUs in manifesting their protective phenotypes.

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Differential Roles of Tau Class Glutathione S-Transferases in Oxidative Stress
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