The yeast Saccharomyces cerevisiae contains two glutaredoxins, encoded by GRX1 and GRX2, that are required for resistance to reactive oxygen species. We recently reported that Grx1 is active as a glutathione peroxidase and can directly reduce hydroperoxides (Collinson, E. J., Wheeler, G. L., Garrido, E. O., Avery, A. M., Avery, S. V., and Grant, C. M. (2002) J. Biol. Chem. 277, 16712–16717). We now show that Grx2 is also a general hydroperoxidase, and kinetic data indicate that both enzymes have a similar pattern of activity, which is highest with hydrogen peroxide, followed by cumene hydroperoxide and tert-butyl hydroperoxide. Furthermore, both Grx1 and Grx2 are shown to be active as glutathione S-transferases (GSTs), and their activity with model substrates such as 1-chloro-2,4-dinitrobenzene is similar to their activity with hydroperoxides. Analysis of the Grx1 active site residues shows that Cys-27, but not Cys-30, is required for both the peroxidase and transferase activities, indicating that these reactions proceed via a monothiol mechanism. Deletion analysis shows that Grx1 and Grx2 have an overlapping function with yeast GSTs, encoded by GTT1 and GTT2, and are responsible for the majority of cellular GST activity. In addition, multiple mutants lacking GRX1, GRX2, GTT1, and GTT2 show increased sensitivity to stress conditions, including exposure to xenobiotics, heat, and oxidants. In summary, glutaredoxins are multifunctional proteins with oxidoreductase, peroxidase, and GST activity, and are therefore ideally suited to detoxify the wide range of xenobiotics and oxidants that can be generated during diverse stress conditions.

Glutaredoxins are small, heat-stable oxidoreductases, first discovered in Escherichia coli as GSH-dependent hydrogen donors for ribonucleotide reductase (1). They have proposed roles in many cellular processes, including protein folding and regulation, reduction of dehydroascorbate, protection against reactive oxygen species and sulfur metabolism (2–4). Glutaredoxins form part of the glutaredoxin system, comprising NADPH, GSH, and glutathione reductase, which transfers electrons from NADPH to glutaredoxins via GSH (5).

Yeast contains two classic glutaredoxin genes, designated GRX1 and GRX2, that share 40–52% identity and 61–76% similarity with those from bacterial and mammalian species (6). Strains deleted for both GRX1 and GRX2 are viable but lack heat-stable oxidoreductase activity using β-hydroxyethyl disulfide (HED)¹ as a model disulfide substrate. Previous studies have shown that the yeast glutaredoxins are active as antioxidants and are required for protection against reactive oxygen species. Mutants lacking GRX1 and GRX2 are sensitive to oxidative stress conditions (6, 7), and overexpression of GRX1 or GRX2 increases resistance to various hydroperoxides (8). Furthermore, expression of GRX1 and GRX2 is up-regulated in response to stress conditions, including exposure to oxidants, with both genes regulated via stress-responsive elements (9). Differences in gene expression between GRX1 and GRX2 indicate that the two glutaredoxins may play distinct roles during different growth and stress conditions (9). Interestingly, a further difference between Grx1 and Grx2 is shown by recent findings indicating that two isoforms of Grx2 can be detected in cells that localize to the cytosol and mitochondria, respectively (10).

Purified Grx1 was shown to have glutathione peroxidase activity and could reduce hydroperoxides directly, including hydrogen peroxide (H₂O₂), tert-butyl hydroperoxide, and cumene hydroperoxide (CHP) (8). This reaction proceeds in a catalytic manner using reducing power provided by NADPH, GSH, and glutathione reductase. The glutathione peroxidase activity of yeast Grx1 seems to be conserved; a recent report has shown that a rice glutathioxin has the same activity (11). Interestingly, yeast does not contain any classic glutathione peroxidases but expresses three phospholipid hydroperoxide glutathione peroxidases (PHGPx) encoded by GPX1–3 (12). These PHGPx enzymes have activity with phospholipid hydroperoxides as well as nonphospholipid peroxides and are able to protect membrane lipids against peroxidation. Thus, Grx1 and Grx2 provide an overlapping defense system with Gpx1–3 protection against a range of hydroperoxides.

The glutaredoxin-mediated resistance to hydroperoxides seems to be part of the glutathione conjugation/removal system of cells, because it is absent in strains lacking glutathione S-transferases (GTT1, GTT2) or the GS-X pump (YCF1) (8). Glutathione S-transferases (GSTs) are a major family of proteins involved in the detoxification of many xenobiotics compounds (13). They catalyze the conjugation of electrophilic substrates to glutathione before their removal from cells via glutathione conjugate pumps. Yeast Gtt1 and Gtt2 share only limited homology with GSTs from other organisms but are active in a GST assay using the model substrate 1-chloro-2,4-dinitrobenzene (CDNB) (14). Strains lacking GTT1 and GTT2 are viable and are unaffected in growth during normal aerobic conditions. In addition, the gtt1 gtt2 mutant does not show any increased sensitivity to CDNB, which is surprising given that Gtt1 and Gtt2 would be expected to detoxify CDNB via conjuga-
Cloning into pGEM-T (Promega), before digestion with NcoI and NheI restriction sites. The resulting 330-bp fragment was sequenced (H11032). In this study, we have extended these findings to show that Grx2, like Grx1, is a general hydroperoxidase. Furthermore, both Grx1 and Grx2 are shown to have glutathione (GSH) transferase activity, and we provide the first evidence that glutathionylated and glutathione S-transferases have overlapping functions in protection against stress conditions including heat shock, oxidative stress, and exposure to CDNB.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Growth Conditions**—The Saccharomyces cerevisiae strains used in this study are described in Table I. Strains were grown in rich YEPD medium (2% (w/v) glucose, 2% (w/v) bactopeptone, and 1% (w/v) yeast extract) or minimal SD medium (0.17% (w/v) yeast nitrogen base without amino acids, 5% (w/v) ammonium sulfate, and 2% (w/v) glucose) supplemented with appropriate nitrogen base without amino acids, 5% (w/v) ammonium sulfate, and 2% (w/v) glucose supplemented with appropriate amino acids and bases: 2 mM leucine, 0.3 mM histidine, 0.4 mM tryptophan, 1 mM lysine, 0.15 mM adenine, and 0.2 mM uracil. Media were solidified by the addition of 2% (w/v) agar.

**Plasmids**—Multicopy plasmids containing GRX1 (mcGRX1) and GRX2 (mcGRX2) have been described previously (8). Plasmid pBAD-GRX1 contains a His6-tagged version of GRX1 and was a kind gift from Barry Rosen (16). The GRX2 gene was amplified from mcGRX2 by PCR to introduce an NcoI site at the 5‘-end and an EcoRI site at the 3‘-end. The forward primer was 5‘-CATTCGTCGCATCCAAAACGTACGCTTCGTCGCATCCAAAACGTAC-3‘ and the reverse primer was 5‘-ATTTGAATTCTTTGTTTGCCTGCAGCCCATCAC-3‘. The resulting 330-bp fragment was cloned into pGEM-T (Promega), before digestion with NcoI and EcoRI and insertion into the Neol and EcoRI sites of pBAD/Myc-HistC (Invitrogen) in frame with the His tag, creating plasmid pBAD-GRX2.

**Site-directed Mutagenesis of GRX1**—The two active site cysteine residues of Grx1 (Cys-27 and Cys-30) were mutated to serine residues in plasmid mcGRX1 creating plasmids mcsgrx1-C27S and mcsgrx1-C30S. PCR was performed using forward mutagenic oligonucleotides that hybridized 53 bp downstream of the ATG start codon (5‘-AAGGAAGAATCTTCTGCGCAACAAAATCGTATCCCTGACATCGAGG-3‘ and 5‘-AAGGAAGAATCTTCTGCGCAACAAAATCGTATCCCTGACATCGAGG-3‘) and a reverse oligonucleotide that hybridized 344 bp downstream of the TAG stop codon (5‘-ATAGCGGTACCCGCATCACGCGATG-3‘). The resulting mutagenized 612-bp fragments were cloned into mcGRX1 using a naturally occurring BglII restriction site and a NheI restriction site introduced by the 3‘ oligonucleotide (underlined).

**Sensitivity to Xenobiotics**—Sensitivity to xenobiotics was determined by spotting strains onto YEPD plates containing various concentrations of H2O2, CHP, and CDNB. Cells were grown to stationary phase, and 5-μl aliquots of diluted cultures were spotted onto appropriate plates. Sensitivity was determined by comparison of growth with the wild-type strain after 3 days. Dose-response curves to H2O2 and CHP were made by growing cells to exponential phase (A600nm = 1) in SD medium at 30 °C, and treating with oxidants for 1 h. Aliquots of cells were diluted in fresh YEPD medium and plated in triplicate on YEPD plates to obtain viable counts after 3 days’ growth.

**Heat Shock Treatment**—Cells were grown to stationary phase in YEPD medium at 30 °C, before heat shock at 52 °C for 10 min. Survival was determined by plating onto YEPD plates in triplicate to obtain viable counts after 3 days’ growth.

**Protein Purification and Western Blot Analysis**—His-tagged Grx1 and Grx2 were purified using Ni2+ agarose columns as described previously (16). Protein extracts were electrophoresed under reducing conditions on 18% SDS-PAGE mini-gels, and Western blot analysis was performed as described previously (8). Protein extracts were electrophoresed under reducing conditions on 18% SDS-PAGE mini-gels, and Western blot analysis was performed as described previously (8).

**Enzyme Assays**—Glutaredoxin activity was measured by the reduction of the mixed disulfide formed between HED and GSH (17). The components of the glutaredoxin system, NADPH (0.4 mM), GSH (1 mM), and glutathione reductase (6 μg/ml), as well as HED (0.7 mM), were added to a reaction volume of 200 μl in 0.1 x Tris HCl, pH 7.4. A mixed disulfide between HED and GSH is formed within 2 min, and the reaction was started by the addition of 5 pmol of Grx1 or Grx2. The reaction was followed by the decrease in A340 because of the oxidation of NADPH.

Peroxidase activity was measured with purified glutaredoxins as described previously (8). Glutathione S-transferase activity was measured with purified glutaredoxins and cell-free extracts using CDNB, 1,2-dichloro-4-nitrobenzene (DCNB), and ethacrynic acid as substrates. Reaction mixtures contained 0.1 mM potassium phosphate buffer, pH 6.5, 1 mM GSH, and varying concentrations of substrate in a reaction volume of 200 μl. Reactions were started by the addition of 5 pmol of purified glutaredoxin or 75 μg of cell-free extracts and were followed by the increase in A340 (CDNB and DCNB) or A254 (ethacrynic acid) caused by the formation of GSH S-conjugates (18).

**RESULTS**

**Glutathione S-transferase Activity of Grx1 and Grx2**—After our observation that Grx1 has glutathione peroxidase activity, we purified Grx2 to compare its catalytic activity. Glutaredoxins are routinely assayed for their ability to reduce the mixed disulfide formed between HED and GSH (17). The oxidoreductase activity of Grx2 (99.6 ± 7.9 μmol/min/mg) was somewhat lower than that of Grx1 (211.8 ± 6.9 μmol/min/mg), indicating that Grx1 is more efficient than the thioredoxin. We next examined whether Grx2 could act as a glutathione peroxidase, similar to Grx1 (8). Grx2 was able to reduce CHP in a catalytic manner in a reaction that depended on the presence of GSH, Gr1, and NADPH (data not shown). The ability of Grx2 to act as a general hydroperoxidase was investigated using various concentrations of hydroperoxides (tert-butyl hydroperoxide, CHP, and H2O2). Grx2 could reduce all three hydroperoxides, and peroxidase activity was hyperbolic with respect to the concentration of peroxide used (data not shown). Comparing the kinetic constants of Grx1 and Grx2 (Table II) showed that Grx1 is catalytically more efficient than Grx2 as a general hydroperoxidase. The Kcat/Km values for Grx2 (0.87–2.2 mM) were generally higher than for Grx1 (0.52–0.88 mM), indicating that Grx1 has a higher affinity for hydroperoxides than Grx2. However, both enzymes showed similar patterns of enzyme specificity (Kcat/Km); the greatest activity was directed toward H2O2, followed by CHP and tert-butyl hydroperoxide.

**Overexpression of GRX1 and GRX2 Increases Sensitivity to CDNB**—Glutathione S-transferases from mammalian systems can act as general hydroperoxidases catalyzing the breakdown of alkyl hydroperoxides to their corresponding alcohols (19).

---

**Table I. Yeast strains used in this study**

| Strain | Genotype | Ref./ source |
|--------|-----------|--------------|
| CY4    | MATa ura3-52 leu2-3, 112 trp1-1 ade2-1 his3-11 can1-100 | 6 |
| Y117   | As in strain CY4 but grx1::LEU2 grx2::HIS3 | 6 |
| Y536   | As in strain CY4 but gt1::TRP1 gt2::URA3 | This study |
| Y781   | As in strain CY4 but grx1::LEU2 grx2::HIS3 gt1::TRP1 gt2::URA3 | This study |
| BY4742 | MATa his3D1 leu2D0 lys2D0 ura3D0 | EUROSCARF* |
| Y14069 | As in strain BY4742 but yeY1::kanMX4 | EUROSCARF |

* EUROSCARF, European Saccharomyces cerevisiae Archive for Functional Analysis.
 Yeast Gtt1 and Gtt2 share little homology with mammalian glutathione S-transferases, and a gtt1 gtt2 mutant is unaffected in sensitivity to the model GST substrate CDNB (14). We therefore examined whether Grx1 and Grx2 might function in the detoxification of CDNB. Overexpression of GRX1 and GRX2 was achieved using multicopy plasmids and was confirmed by means of Western blot analysis (8). Wild-type and ycf1 mutant transformants were grown to stationary phase and spotted onto plates containing various concentrations of CDNB. No strains were able to grow on plates containing CDNB concentrations higher than 0.1 mM (data not shown). Surprisingly, elevating the level of Grx1 or Grx2 was found to increase the sensitivity of both strains to 0.075 mM CDNB compared with the vector-alone controls (Fig. 1). This is similar to findings that glutaredoxins increase the sensitivity of a ycf1 mutant to CHP (8), and rat liver GSTT1 causes increased bioactivation of dialkylalkanes (20). These results indicate that glutaredoxins have some activity toward CDNB and may convert it to a more toxic product. We therefore examined whether the yeast glutaredoxins could act as glutathione S-transferases.

Glutathione S-transferase Activity of Grx1 and Grx2—The ability of glutaredoxins to catalyze the formation of GSH conjugates was tested in vitro using purified Grx1 and Grx2. Reaction mixtures contained GSH and CDNB and were followed by the increase in absorbance at 340 nm caused by the formation of the conjugate. Both Grx1 and Grx2 were able to catalyze conjugate formation, and reactions were dependent on the presence of both GSH and glutaredoxins because there was little or no activity when each was omitted from the assay (Fig. 2A). The conjugation of GSH to CDNB was analyzed using differing amounts of Grx1 and Grx2 and was found to show strict linearity, confirming that glutaredoxins have efficient catalytic activities as glutathione S-transferases (Fig. 2B). We next determined the kinetics of glutathione S-transferase activity with GST substrates, including CDNB, DCNB, and ethacrynic acid. Grx1 and Grx2 showed no measurable activity with ethacrynic acid, whereas both enzymes were active against CDNB and DCNB (Table II). Comparing the kinetic constants (Table II) showed that Grx2 is catalytically more efficient than Grx1 as a glutathione S-transferase. *Km* values for Grx1 (1.5–4.2 mM) were generally higher than for Grx2 (0.99–1.1 mM), and enzyme specificity values (Kcat/Km) for Grx2 (4.2–5.9 × 10^3 M^−1 s^−1) were higher than for Grx1 (1.7 × 10^2 to 3.1 × 10^3 M^−1 s^−1).

Cellular Glutathione S-transferase Activity—Given that purified recombinant Gtt1 and Gtt2 (14) and Grx1 and Grx2 (Fig. 2) have glutathione S-transferase activity, we determined their relative contributions to cellular GST activity. The quadruple gtt1 gtt2 grx1 grx2 mutant was viable and was unaffected in growth during normal aerobic conditions compared with the wild-type strain (data not shown). GST activity was therefore assayed in cell-free lysates from the wild-type, gtt1 gtt2, grx1 grx2, and gtt1 gtt2 grx1 grx2 mutants using CDNB and DCNB as substrates (Fig. 3). The wild-type strain showed similar GST activity with both CDNB and DCNB. Loss of GTT1 and GTT2 had little effect on GST activity assayed with CDNB, and caused a slight increase in activity toward DCNB. In contrast, loss of GRX1 and GRX2 reduced the cellular GST activity with CDNB and DCNB by ~50%. GST activity measured from the quadruple mutant was ~8-fold lower than the wild-type strain with both CDNB and DCNB. These data indicate that Grx1, Grx2, Gtt1, and Gtt2 account for the majority of glutathione S-transferase activity detected within yeast cells, and glutare-

| Substrate | Grx1 | Grx2 |
|-----------|------|------|
|           | Kcat | Km  | Kcat/Km | Kcat | Km  | Kcat/Km |
| H2O2      | 0.88 | 47.1| 15.3 x 10^4| 2.0  | 52.9| 2.6 x 10^4 |
| t-BH       | 0.37 | 2.9 | 7.8 x 10^3 | 2.2  | 14.3| 6.5 x 10^3 |
| CHP        | 0.52 | 14.3| 2.8 x 10^4 | 0.57 | 12.9| 1.5 x 10^4 |
| CDNB       | 4.2  | 13.1| 3.1 x 10^3 | 0.17 | 0.99| 5.9 x 10^3 |
| DCNB       | 1.3  | 0.22| 1.7 x 10^2 | 0.27 | 1.1 | 4.2 x 10^3 |

* K values for each substrate were calculated using Eadie Hofstee plots and represent averages of at least three independent determinations.

**Fig. 1.** Overexpression of GRX1 and GRX2 increases sensitivity to CDNB. Sensitivity was determined by spotting strains onto plates containing various concentrations of CDNB. Cultures of wild-type and ycf1 mutant strains containing YEp24, mcGRX1, and mcGRX2 were grown into stationary phase and diluted (A_{600} = 1, 0.5, and 0.1) before spotting onto appropriate plates. Plates were incubated at 30 °C for 3 days before scoring growth. Results are shown for plates containing no CDNB (YEPD) and 0.075 mM CDNB.

**Fig. 2.** Glutathione S-transferase activity of Grx1 and Grx2. A, glutathione S-transferase activity of Grx1 and Grx2 was measured in vitro using purified Grx1 and Grx2. The components of the complete reaction mixture were GSH (0.1 mM) and CDNB (0.3 mM) in 0.1 mM potassium phosphate buffer, pH 6. Reactions were started by the addition of 5 pmol of Grx1 or Grx2 and followed by an increase in absorbance (A_{340}) of GST activity dependent on the presence of glutaredoxin and GSH; minimal activity was detected in reactions in which each component was omitted individually. B, GST activity with CDNB catalyzed by Grx1 and Grx2 was found to show strict linearity at concentrations ranging from 5 to 25 pmol.
doxins seem to be more active than glutathione S-transferases under the conditions tested.

**Overlapping Functions of Yeast Glutaredoxins and Glutathione S-transferases**—Strains deleted for GTT1 and GTT2 are unaffected in growth during normal aerobic conditions but exhibit increased sensitivity to heat shock in stationary phase (14). We therefore investigated the heat shock sensitivity of the quadruple gtt1 gtt2 grx1 grx2 mutant. Cells were grown to stationary phase at 30 °C and subjected to heat shock at 52 °C for 10 min. This treatment resulted in 35% loss of viability in the wild-type strain (Fig. 4A). Stains deleted for GRX1 and GRX2 were no more sensitive to heat shock than the wild-type strain. In agreement with previous findings (14), the gtt1 gtt2 mutant was sensitive compared with the wild-type strain, with −54% survival after the heat treatment. Interestingly, the quadruple gtt1 gtt2 grx1 grx2 mutant was even more sensitive, and survival was reduced to −36%.

Mutants lacking GRX1 and GRX2 are sensitive to oxidative stress conditions (6), whereas mutants lacking GTT1 and GTT2 are unaffected in sensitivity to a range of oxidants and xenobiotics (14). We therefore compared the gtt1 gtt2, grx1 grx2, and gtt1 gtt2 grx1 grx2 mutants for sensitivity to stress conditions. Cells were grown to stationary phase and spotted onto plates containing various concentrations of H2O2, CHP, and CDNB (Fig. 4B). For all three treatments, the quadruple gtt1 gtt2 grx1 grx2 mutant was more sensitive than either the wild-type or gtt1 gtt2 and grx1 grx2 mutant strains. These data indicate that glutaredoxins (Grx1–2) and glutathione S-transferases (Gtt1–2) have an overlapping function in protection against stress conditions induced by heat, oxidants, and CDNB.

Cys-27, but Not Cys-30, Is Required for Peroxidase and Transferase Activity of Grx1—The structure of glutaredoxins has been highly conserved throughout evolution, particularly in the region of the active site (3, 17) including two redox-active cysteine residues (positions 27 and 30 in yeast numbering (6)). The two active site Cys residues in Grx1 were replaced with Ser residues to determine their role in peroxidase and transferase activities. These mutations were constructed in plasmid YEp24, mcGRX1, mcGRX1-C27S, and mcGRX1-C30S, respectively (see “Experimental Procedures”). Peroxidase activity was tested by examining resistance to H2O2 and transferase activity by resistance to CDNB. Overexpression of GRX1 and grx1-C30S increased the resistance of a wild-type strain to H2O2, whereas grx1-C30S had no effect relative to the vector control (Fig. 5A). Similarly, overexpression of GRX1 and grx1-C30S increased the sensitivity of a wild-type strain to CDNB, whereas, grx1-C30S had no effect relative to the vector control.
mutants lacking levels of protein-GSH mixed disulfides are unaffected in yeast in vitro. Mixed disulfides formed with GSH (25). Mixed disulfides can against oxidative stress by catalyzing the reduction of protein tively (10). Glutaredoxins have long been thought to protect complementary roles in the cytoplasm and mitochondria, respec- 

the mitochondria may account for the apparent redundancy of glutaredoxins are multifunctional enzymes with oxidoreduc-

the Ras-protein kinase A pathways (9). Our studies show that activated by multiple stress conditions via the Hog1 MAPK and GRX1

sponses to a diverse range of stress conditions, including oxi-

larities among the many sets of genes activated in response to differ- ent stress conditions (22). For example, the cellular re-

number of conserved residues that are mainly clustered in the

GSTO 1

–

GSTO 1

–

1, and

–

1 and

–

human GSTO 1

–

32. In the present study, we show that Cys-27, but not Cys-30, is required for both the peroxidase and transferase activities, indicating that these re-

2 and Grx2 have GST activity. Both Grx1 and Grx2 were active

GSTS with Gtt1 and Gtt2, with loss of all four genes substanc-

Grx1 and Grx2 and human Grx1. Members of the second subfamily of glutaredoxins, which differ from classical glutaredoxins in that they contain a single cysteine residue at their active sites, were originally identified in yeast (GRX3–5) but are conserved throughout evolution from bacte-

Grx2 has revealed that these glutaredoxins are homologous to the GST superfamily of proteins (33). This structural homology is particularly shown toward enzymes belonging to the θ- and ω-classes of GSTs.

Sequence analysis of yeast Grx1 and Grx2 reveals significant homology with the ω-class of GSTs (Fig. 6). Comparison with human GSTO 1–1 and Schistosoma mansoni GSTO1 identifies a number of conserved residues that are mainly clustered in the N-terminal portion of Grx1 and Grx2. Identical residues in- clude the Cys-Pro active site residues of the yeast glutaredoxins. The corresponding Cys residue in ω-class GSTs (Cys-32 in GSTO 1–1 and Cys-25 in SmGSTO1) has been shown to form part of the glutathione binding site (the G-site), which makes a mixed disulfide with GSH (29, 34). Glutathione S-transferases

Fig. 6. Alignment of the amino acid sequences of Grx1 and Grx2 with ω-class GSTs. The amino acid sequences of Grx1, Grx2, human GSTO 1–1, and Schistosoma mansoni GSTO1 were aligned using ClustalW (www.ebi.ac.uk/clustalw) and displayed using GeneDoc (www.psc.edu/biomed/genedoc/). The sequences are aligned for maximal homology; dashes are used to denote gaps introduced for maximal alignment. Residues that are identical in all four sequences are denoted with an asterisk. Residues that are conserved in all four sequences are boxed in black shading, and residues that are conserved in at least three sequences are boxed in gray shading.

DISCUSSION

Yeast, like all organisms, must be able to adapt to changes in their external environment, such as exposure to xenobiotics and oxidants. In most cases, these responses depend on gene activation, such as the response to H$_2$O$_2$, where a substantial number of proteins is induced (21). Furthermore, genome-wide analysis has shown that there are differences as well as similarities among the many sets of genes activated in response to different stress conditions (22). For example, the cellular responses to a diverse range of stress conditions, including oxidative, osmotic, heat, and starvation, are mediated by a number of genes containing stress-responsive elements (23, 24). GRX1 and GRX2 form part of these responses, with both genes activated by multiple stress conditions via the Hog1 MAPK and the Ras-protein kinase A pathways (9). Our studies show that glutaredoxins are multifunctional enzymes with oxidoreductase, peroxidase, and GST activity and are therefore ideally suited to detoxify the wide range of xenobiotics and oxidants that might be generated during diverse stress conditions.

The antioxidant activity of Grx1 and Grx2 provides protection against hydroperoxides (6, 8). The localization of Grx2 in the mitochondria may account for the apparent redundany of yeast glutaredoxins, indicating that Grx1 and Grx2 play com-

componentary roles in the cytoplasm and mitochondria, respective- 

Glutaredoxins have long been thought to protect against oxidative stress by catalyzing the reduction of protein mixed disulfides formed with GSH (25). Mixed disulfides can protect protein-SH groups against irreversible oxidation, and in vitro studies have shown that they can be reduced by gluta-

However, in vivo data indicate that the levels of protein-GSH mixed disulfides are unaffected in yeast mutants lacking GRX1 and GRX2 during both normal growth conditions and exposure to H$_2$O$_2$ (6, 28). In this present study, we show that both Grx1 and Grx2 have oxidoreductase activity against the mixed disulfide formed between HED and GSH, although it remains unclear whether this activity serves a protective function against physiological substrates formed during stress conditions.

We originally showed that Grx1 is a glutathione peroxidase that can reduce various hydroperoxides with activity compara-

that of yeast PHGPs2 and various eukaryotic glutathione peroxidases (8). Similarly, in the present study, we show that Grx2 can directly reduce hydroperoxides, albeit with a lower catalytic efficiency than Grx1. Because glutathione peroxidase activity can be associated with glutathione S-transferases in mammalian cells (19), we analyzed whether Grx1 and Grx2 have GST activity. Both Grx1 and Grx2 were active in a GST assay using CDNB and DCNB as substrates, with Grx2 showing a higher catalytic efficiency than Grx1. Furthermore, the activity of Grx1 and Grx2 with GST substrates was similar to their activity with hydroperoxides. Mutant analysis revealed that Grx1 and -2 have an overlapping function as GSTs with Gtt1 and Gtt2, with loss of all four genes substantially reducing cellular GST activity and leading to sensitivity to stress conditions, including exposure to xenobiotics, heat, and oxidants.

Two reaction mechanisms have been described for glutare-

GST superfamily of proteins (33). This structural homology is particularly shown toward enzymes belonging to the θ- and

Based on structural and functional data, three glutaredoxin subfamilies have been proposed (33). Yeast Grx1 and Grx2 are part of the first class, which includes classic dithiol glutaredoxins, such as E. coli Grx1 and Grx2 and human Grx1. Members of the second subfamily of glutaredoxins, which differ from classical glutaredoxins in that they contain a single cysteine residue at their active sites, were originally identified in yeast (GRX3–5) but are conserved throughout evolution from bacte-

Downloaded from http://www.jbc.org/ on July 21, 2018
contain a substrate binding site (H-site), adjacent to the G-site, that allows conjugation of the thiol group of GSH to an electrophile. This site is constructed from elements of both the N- and C-terminal portions of GSTs, and differences in substrate specificity are thought to be caused by differences in the confirmation of the H-site. The H-site in GSTO 1–2 (29) is a well-defined cavity that includes Pro-33 (Pro-28 in Grx1 and Grx2) and Phe-31 (Tyr-26 in Grx1 and Grx2) flanking the catalytic Cys-32 residue. The ω-class of GSTs are multifunctional enzymes that have activity with a broad range of substrates (13, 34). For example, human GSTO1–1 exhibits GST activity, glutathione-dependent peroxidase activity, and dehydroascorbate activity characteristic of glutaredoxins (29).

The range of glutathione-dependent antioxidants that has been described in yeast differs from that in other eukaryotic organisms. Unusually, yeast expresses three PHGPxs in the absence of conventional glutathione peroxidases (12). In addition, only two GST isoenzymes have been found in yeast, which share little homology with GSTs from other organisms (14). Our observations suggest that the multiple antioxidant activities of Grx1 and Grx2 have evolved to complement the peroxidase activities of PHGPx1–3 and the transferase activities of Gtt1–2 in protection against stress conditions. In this view, glutaredoxins are able to protect against a wide range of toxic compounds (xenobiotic or endobiotic) to which yeast cells might be exposed. It remains to be determined whether glutaredoxins from other eukaryotes have a similar range of biochemical activities, but the peroxidase activity seems to be a conserved activity for at least one plant glutaredoxin (11).

Acknowledgments—We are grateful to Barry Rosen for donating plasmid pBAD-YGRX1 and Ales Vancura for the gtt1 and gtt2 mutants.

REFERENCES

1. Holmgren, A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 2275–2279
2. Holmgren, A. (1989) J. Biol. Chem. 264, 13963–13966
3. Wells, W. W., Yang, Y., and Deits, T. L. (1993) Adv. Enzymol. 66, 149–199
4. Chrestensen, C. A., Eckman, C. B., Starke, D. W., and Mieyal, J. J. (1995) FEBS Lett. 374, 25–28
5. Holmgren, A. (1990) in Glutathione: Metabolism and Physiological Functions (Vina, J., ed) pp. 146–154, CRC Press Inc, Boca Raton
6. Luikenhuis, S., Dawes, I. W., and Grant, C. M. (1997) Mol. Biol. Cell 9, 1081–1091
7. Rodriguez-Manzaneque, M. T., Ros, J., Cabioch, E., Sorribas, A., and Herrero, E. (1999) Mol. Cell. Biol. 19, 8180–8190
8. Collinson, K. J., Wheeler, G., Garrido, E. O., Avery, A. M., Avery, S. V., and Grant, C. M. (2002) J. Biol. Chem. 277, 16712–16717
9. Grant, C. M., Luikenhuis, S., Beckhouse, A., Soderbergh, M., and Dawes, I. W. (2000) Biochim. Biophys. Acta 1490, 33–42
10. Pedrajas, J. R., Porras, P., Martinez-Galisteo, E., Padilla, C. A., Miranda-Vizuete, A., and Barrena, J. A. (2002) Biochem. J. 364, 617–623
11. Lee KO, L. J., Yoo JY, Jang HH, Moon JC, Jung BG, Chi YH, Park SK, Lee SS, Lim CO, Yun DJ, Che MJ, Lee SY. (2002) Biochem. Biophys. Res. Commun. 296, 1153–1156
12. Avery, A. M., and Avery, S. V. (2001) J. Biol. Chem. 276, 33730–33735
13. Sheehan, D., Meade, G., Foley, V. M., and Dowd, C. A. (2001) Mol. Biol. Cell 12, 4241–4257
14. Marchler, G., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., Avasthi, S., and Avasthi, Y. C. (1999) Arch. Biochem. Biophys. 367, 216–224
15. Godon, C., Lagniel, G., Lee, J., Buhler, J.-M., Kieffer, S., Perrot, M., Boucherie, H., Toledano, M. B., and Labarre, J. (1998) J. Biol. Chem. 273, 22480–22485
16. Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Stora, G., Botstein, D., and Brown, P. O. (2000) Mol. Cell. Biol. 11, 4241–4257
17. Marchler, G., Schaller, C., Adam, G., and Ruis, H. (1995) EMBO J. 14, 997–2003
18. Kobayashi, N., and McEntee, K. (1993) Mol. Cell. Biol. 13, 248–256
19. Kohda, M., and Goto, S. (1995) Arch. Biochem. Biophys. 319, 1–9
20. Thomas, J. A., Poland, B., and Honzatkou, D. (1995) Arch. Biochem. Biophys. 335, 61–72
21. Jung, C.-H., and Thomas, J. A. (1996) Arch. Biochem. Biophys. 333, 61–72
22. Garrido, E. O., and Grant, C. M. (2002) Mol. Microbiol. 43, 993–1003
23. Board, P. G., Coggan, M., Chelvanayagam, G., Eastal, S., Erwin, B., Haste, T., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., Rosner, M. H., Chrunyk, B. A., Perregaux, D. E., Gabel, C. A., Georghegan, K. F., and Pandit, J. (2000) J. Biol. Chem. 275, 24798–24806
24. Bushweller, J. H., Amling, F., Wuthrich, K., and Holmgren, A. (1992) Biochemistry 31, 9288–9293
25. Yang, Y. F., and Wells, W. W. (1991) J. Biol. Chem. 266, 12759–12765
26. Kan, Z.-R., Sardana, M. K., Jacobs, J. W., and Polokoff, M. A. (1990) Arch. Biochem. Biophys. 282, 110–115
27. Xia, B., Vlamis-Gardikas, A., Holmgren, A., Wright, P. E., and Dyson, H. J. (1998) J. Biol. Chem. 273, 22480–22485
28. Giradini, J., Amirante, A., Zemzoumi, K., and Serra, R. (2002) Eur. J. Biochem. 269, 5512–5521
Role of Yeast Glutaredoxins as Glutathione S-transferases
Emma J. Collinson and Chris M. Grant

J. Biol. Chem. 2003, 278:22492-22497.
doi: 10.1074/jbc.M301387200 originally published online April 8, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301387200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 14 of which can be accessed free at http://www.jbc.org/content/278/25/22492.full.html#ref-list-1