A Novel Membrane-bound Glutathione S-Transferase Functions in the Stationary Phase of the Yeast Saccharomyces cerevisiae*  

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The glutathione S-transferases (GSTs) represent a significant group of detoxification enzymes that play an important role in drug resistance in all eukaryotic species. In this paper we report an identification and characterization of the two Saccharomyces cerevisiae genes, GTT1 and GTT2 (glutathione transferase 1 and 2), coding for functional GST enzymes. Despite only limited similarity with GSTs from other organisms (~50%), re-combinant Gtt1p and Gtt2p exhibit GST activity with 1-chloro-2,4-dinitrobenzene as a substrate. Both Gtt1p and Gtt2p are able to form homodimers, as determined by two hybrid assay. Subcellular fractionation demonstrated that Gtt1p associates with the endoplasmic reticulum. Expression of GTT1 is induced after diauxic shift and remains high throughout the stationary phase. Strains deleted for GTT1 and/or GTT2 are viable but exhibit increased sensitivity to heat shock in stationary phase and limited ability to grow at 39 °C.

Cellular metabolism and detoxification of xenobiotics (carcinogens, toxins, environmental pollutants, and drugs) occur in three stages. In stage I, toxins are activated by oxidation, reduction, or hydrolysis to introduce a functional group. In stage II, the functional group is conjugated with glutathione (GSH), glucuronic acid, or glucose. In particular, S-conjugates of GSH are formed by cysteolic glutathione S-transferase (GST) enzymes. In stage III, GSH conjugates are eliminated from the cytosol by a GS-X pump located in the plasma membrane of animal cells and the vacuolar membranes of yeast cells (1).

The level of expression of GSTs and their biochemical properties are crucial factors in determining cellular resistance to carcinogens, antitumor drugs, environmental pollutants, and products of oxidative stress (2–5). All eukaryotes possess multiple GST isoenzymes, each displaying distinct catalytic as well as noncatalytic binding properties (6, 7). The functional basis for the catalytic activity of GSTs is their ability to bind GSH and lower the pK of its sulfhydryl group (–SH) from 9.0 to about 6.5. Once the thiolate anion (–GS) is formed in the binding site for GSH, it is capable of reacting spontaneously by nucleophilic attack with electrophilic xenobiotics in close proximity. Therefore, catalysis by GST occurs through the combined ability of the enzyme to (i) bind GSH and promote the formation of –GS, and (ii) bind hydrophobic electrophilic compounds at a nearby site. In contrast to the GSH-binding site, which exhibits a high specificity, the second substrate-binding site displays a broad specificity toward hydrophobic compounds (8).

The yeast Saccharomyces cerevisiae is an excellent model for studying detoxification pathways due to the ease of its genetic manipulation. Indeed, a large number of genes mediating the resistance to toxic xenobiotics have been identified in yeast. The gene products described so far fall into two major classes: (i) membrane transport proteins belonging to the ATP-binding cassette (ABC) superfamily, such as Snq2p, Pdr5p, and Ycf1p, and (ii) factors regulating the expression of these membrane transport proteins, including Pdr1p, Pdr5p, Pdr7p, yAP1, and yAP2 (9).

The detoxification pathways involving GST enzymes have not been studied in yeast, because gene(s) coding for GST in S. cerevisiae have not been identified (10–12). However, overexpression of Issatchenkia orientalis GST in S. cerevisiae elevated the resistance to o-dinitrobenzene (10), and expression of human Alpha or Pi GST in S. cerevisiae resulted in a marked increase in resistance to the anticancer drugs adriamycin and chlorambucil (13). The GSH conjugates are transported to the vacuole by the yeast GS-X pump Ycf1p (product of YCF1 gene; Refs. 14–16).

In this paper we report an identification of the first two S. cerevisiae genes, GTT1 and GTT2, coding for functional GST enzymes. We expressed both Gtt1p and Gtt2p in E. coli, purified the proteins, and determined that they both exhibit GST activity. Transcription from GTT1 promoter is induced by osmotic stress and xenobiotics, and, most significantly, after diauxic shift. Subcellular fractionation experiments show that Gtt1p associates with the endoplasmic reticulum. GTT1 and GTT2 are not essential to the cell, but gtt1Δ, gtt2Δ, and gtt1Δgtt2Δ mutant strains exhibit significantly reduced thermotolerance in the stationary phase and limited ability to grow at 39 °C.

EXPERIMENTAL PROCEDURES  

Strains and Media—Yeast strains used in this study (listed in Table I) were grown in rich medium (YPD; 1% yeast extract, 2% Bacto-peptone, 2% glucose) or under selection in synthetic minimal medium (SD) supplemented with appropriate nutrients. Yeasts were manipulated as described previously (17).

Plasmids—Plasmid pYX242-HT-T7 was constructed by ligating NcoI-SmaI fragment from plasmid pET28 (Novagen) into NcoI-SmaI-digested pYX242 plasmid (Novagen; yeast shuttle plasmid with 2µ, LEU2, and TPI [thiophosphate isomerase] promoter). This introduces His tag and T7 epitope at the N terminus of a protein expressed from this plasmid. The coding region of GTT1 was isolated by PCR amplification using total yeast (strain W303-1a) DNA as a template and Vent DNA polymerase. The GTT1–5A oligonucleotide (5’-GCGCGAGCTCTTATTGGGCC-3’) was isolated by PCR amplification using total yeast (strain W303-1a) DNA as a template and Vent DNA polymerase. The GTT1–3A oligonucleotide (5’-GGGAGCTCTTATTGGGCC-3’) was isolated by PCR amplification using total yeast (strain W303-1a) DNA as a template and Vent DNA polymerase. The GTT1–3A oligonucleotide (5’-GGGAGCTCTTATTGGGCC-3’).
CTGAAATTTGCTACCTAAAGACGGC-3') introduced a SacI site. The resulting 0.7-kb DNA fragment was digested with BamHI and SacI and ligated in pX242-HT-T7 or pCITE-4C plasmids restricted with the same enzymes to yield pX242-HT-T7-GTT1 or pCITE-4C-GTT1, respectively.

For construction of pET23b-GTT1, pET14b-GTT1, pGB9-TGT1, and pGAD424-GTT1, the GTT1 coding sequence was generated by PCR as described above with oligonucleotides GTT1–5B (5'–GGCGCGATCATATGCTGAGGAAATTGCTACCTAAAGCACGC-3') respectively. The resulting 0.7-kb DNA fragment was digested with NdeI and BamHI sites and GTT1–3B introduces Xhol and BamHI sites. The PCR-derived GTT1 fragment was digested with NdeI and XhoI and ligated in NdeI/XhoI sites of pET23b (Novagen) and in NdeI-BamHI sites of pET14b (Novagen). The resulting plasmids, pET23b-GTT1 and pET14b-GTT1, expressed N- and C-terminal Glipl fusions with His tag in E. coli, respectively. The above GTT1 fragment (generated with GTT1–5B and GTT1–3B primers) was digested with BamHI and ligated in BamHI site of pGB9T and pGAD424, to create pGB9T-GTT1 and pGAD424-GTT1, respectively.

To construct pET23d-GTT2, pGB9T-GTT2, and pGAD424-GTT2, BamHI and Neol sites were first added to the 5' end of the coding region, and XhoI and BamHI sites were added to the 3' end of the coding region by PCR using oligonucleotides GTT2–5 (5'–GGCGCGATCATATGCATATGCTGGTCTAATGGG-3') and GTT1–3B (5'–GGCGGGATCTACCTAAAGACGGC-3') fragment was excised from pJJ248 (19) and ligated in pGB9T and pGAD424, to create pGB9T-GTT2 and pGAD424-GTT2, respectively.

The plasmids for expression and two-hybrid assay with Ure2p were built by first adding BamHI and NdeI sites to the 5' end of the coding region of URE2, and XhoI and BamHI sites to the 3' end of the coding region by PCR with oligonucleotides URE2–5 (5'–GGCGCGATCATATGCGGATATGAACGAGGAGATGGTG-3') and URE2–3 (5'–GGCGGGATCTACCTAAAGACGGC-3'), respectively. The fragment was then digested with NdeI and XhoI and ligated into the same sites of pET23d to construct pET23d-GTT2, or it was digested with BamHI and ligated in BamHI site of pGB9T or pGAD424 to create pGB9T-GTT2-GT and pGAD424-GTT2, respectively.

β-Galactosidase Assays—Strain JC105 was grown in SD-Ura medium at 30 °C overnight and then diluted in YPD medium to an A_{600 nm} of 0.15. Cells were grown to an A_{600 nm} of 1.3 before treatment with various stress conditions for 1 h. In heat shock experiments, cells were grown to 23 °C to an A_{600 nm} of 1.5, and then shifted to 37 °C for 1 h. Cells were harvested and disrupted by vortexing with glass beads, and the β-galactosidase activity was assayed using o-nitrophenyl-β-D-galactoside essentially as described previously (20).

Protein concentration was determined using the Coomassie Plus protein assay kit (Pierce).

Expression and Purification of Glipl, Gli2p, Ure2p, and Ure2pΔN—BL21 (DE3) cells harboring pET23b-GTT1, pET14b-GTT1, pET23d-GTT2, pET23b-ure2p, and pET23b-ure2pΔN were grown in LB medium containing ampicillin (100 μg/ml) at 37 °C to an A_{600 nm} of 0.1, at which point isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 1 mM. After 3 h of induction, cells were harvested by centrifugation (15 min at 3000 x g; 4 °C) and stored at −70 °C until used. All subsequent steps were carried out at 4 °C. Frozen cells were thawed, resuspended in three volumes of lysis buffer (20 mM Tris, pH 7.5, 0.5 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml each of leupeptin, aprotinin, and pepstatin) and ruptured by sonication (10 × 30 s). The homogenate was centrifuged (10 min at 5000 × g; 4 °C), supernatant removed, and the pellet resuspended in the lysis buffer, sonicated (10 × 30 s) and centrifuged again. The
supernatants were combined and centrifuged (1 h at 170,000 × g, 4 °C). The clear supernatant was loaded onto a Ni²⁺-agarose column (2 ml) pre-equilibrated with lysis buffer. The column was washed with 20 ml of the lysis buffer and then eluted with 4-ml aliquots of the lysis buffer containing 10, 20, 50, 100, 200, and 500 mM imidazole. Fractions with high GST activity were pooled and dialyzed against storage buffer (50% glycerol, 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM dithiothreitol, 0.1 mM EDTA, 0.01% Brij 35).

**GST Assay**—The GST activity of Gtt1p and Gtt2p was assayed spectrophotometrically (21). The reaction mixture contained 0.1 mM potassium phosphate buffer, pH 6.5, 1 mM reduced glutathione (GSH), and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction mixture was preincubated at 25 °C (5 min), and the reaction was initiated by an addition of the enzyme. The increase of A₃₄₀ nm was recorded, and the activity was calculated from the difference in A₃₄₀ nm changes in the reaction mixtures with and without the enzyme.

**Subcellular Fractionation and Immunoblotting**—JC104 cells were grown, lysed, and fractionated by differential centrifugation into 10,000 × g pellet (P1), 170,000 × g pellet (P2), and supernatant (S) fractions as described previously (17, 22). P1 and P2 pellets were then subjected to density gradient fractionation (17, 22). The gradients were fractionated from the top (600-μl fractions) and labeled sequentially as fractions 1–20. All fractions were assayed in duplicate for protein and sucrose concentration. Epitope-tagged HT-T7-Gtt1p was assayed by immunoblotting with monoclonal T7-Tag® antibody. The following marker proteins were assayed by immunoblotting with specific antibodies: vanadate-sensitive plasma membrane ATPase (assayed with Pma1 polyclonal antibody provided by Dr. A. Chang), Kex2p (assayed with KXR-B6 polyclonal antibody provided by Dr. R. Fuller), vacuolar H⁺-ATPase subunit (V-ATPase; assayed with 10D7-A7-B2 monoclonal antibody, Molecular Probes), mitochondrial porin (assayed with 16G9-E6 monoclonal antibody, Molecular Probes), dolichophosphate mannosyl synthase (assayed with 5C5-A7 monoclonal antibody, Molecular Probes). Immunoreactivity was measured by enhanced chemiluminescence using sheep anti-mouse or anti-rabbit IgG conjugated to horse-radish peroxidase (Amersham Pharmacia Biotech). Images were collected on x-ray film and quantified by laser densitometry.

**RESULTS**

**Identification of GTT1 as Glutathione S-Transferase**—A Blast search of GenBank and Saccharomyces Genome Database revealed three open reading frames whose putative protein products exhibited similarity to known mammalian and plant GST enzymes. The first uncharacterized open reading frame, YIRO36c, codes for a protein of 234 amino acids (named Gtt1p; glutathione transferase 1). Since Gtt1p exhibits only a limited sequence homology with GSTs from other organisms, it was not clear whether it also encodes a protein with GST activity. When we overexpressed HT-T7-Gtt1p (N-terminal fusion with His tag and T7 epitope) in JC104 strain, the GST activity in the cell lysate was elevated only 2-fold compared with the W303–1a strain. Synthesis of Gtt1p by in vitro transcription-translation yielded similar results, but the GST activity in the reticulocyte lysate was elevated only 50% compared with control. Since these results do not unambiguously show that GTT1 codes for a protein with GST activity, we expressed Gtt1p in E. coli as a C-terminal or N-terminal fusion with His tag. After induction with isopropyl-1-thio-β-D-galactopyranoside, we detected high GST activity in E. coli lysate.

This GST activity co-eluted with the induced protein during a subsequent chromatography on Ni²⁺-agarose. The enzyme characterization was performed with purified Gtt1p with C-terminal His tag. Km values for GSH and 1-chloro-2,4-dinitrobenzene (CDNB; the model substrate for GST) are 0.6 mM and 3.3 mM, respectively, and Vmax is 500 nmol/mg/min. These results confirm that GTT1 codes for a protein with GST activity.

The second gene with sequence similarity to GSTs is also an uncharacterized open reading frame (YLL060c; GenBank Accession number Z73165). Again, after we had confirmed that the protein product of YLL060c exhibits GST activity (described below), we named it GTT2 (glutathione transferase 2).
of Ure2p synthesis can be easily detected on SDS-polyacrylamide gels, it did not result in any detectable GST activity in the E. coli extract. Since Ure2p contains an N-terminal extension that is not shared by Gtt1p, Gtt2p, or other GST enzymes, we constructed, expressed, and purified (Fig. 1) a truncated form of Ure2p (Ure2p(ΔN)), which has this N-terminal extension (93 amino acids) deleted. Ure2p(ΔN) also did not exhibit any detectable GST activity. This result, however, is not entirely surprising. Coschigano and Magasanik (23) and Blinder et al. (24) found that Ure2p directly, and in catalytic fashion, inhibits the ability of zinc finger protein Gln3p to activate transcription. Given the sequence similarity of Ure2p with GST enzymes, these authors speculated about possible Ure2p-catalyzed glutathiolation and inactivation of Gln3p, but they did not detect any GST activity associated with Ure2p.

Pairwise protein sequence alignment (CLUSTAL W program with PAM250 weight table; Ref. 25) revealed that Gtt1p and Gtt2p exhibit only 43% sequence similarity (11% identity and additional 32% similarity), and both Gtt1p and Gtt2p display 38% similarity with Ure2p. Blast search of GenBank identified GST from maize (form III and IV), Silene vulgaris (plant Phi class), Arabidopsis, Manduca sexta, silkworm, and house fly as most similar GST enzymes (about 50% similarity with both Gtt1p and Gtt2p). The conserved amino acid residues are clustered in the N-terminal region (Fig. 2), which corresponds to the GSH binding site (26, 27).

The cytosolic GST enzymes in all vertebrate species comprise two subunits, and exist as either homodimers or heterodimers, with each subunit in the dimeric protein functioning independently (2). To determine whether Gtt1p, Gtt2p, and Ure2p are also able to form homodimers or heterodimers, we performed a two-hybrid assay (28, 29). The coding regions of GTT1, GTT2, and URE2 were inserted in both GALA DNA binding domain plasmid and GAL4 activation domain plasmid, and strains containing both constructs and corresponding control plasmids were streaked on selective medium containing 10 μg/ml 5-amino-1,2,4-triazole (Fig. 3). The results show that both Gtt1p and Gtt2p are able to form homodimers in vivo, but they do not form Gtt1p-Gtt2p, Gtt1p-Ure2p, and Gtt2p-Ure2p heterodimers. We could not test the Ure2p ability to dimerize, since when expressed from GAL4 DNA binding domain plasmid, it nonspecifically activated transcription of the reporter genes.

Subcellular Localization of Gtt1p—Cell lysate, prepared from JC104 strain which expresses HT-T7-Gtt1p fusion, was subjected to differential centrifugation as described previously (17). The amounts of HT-T7-Gtt1p and organelle specific markers in individual fractions were determined by Western blotting and chemiluminescence detection. The majority of Gtt1p (63%) was found in 10,000 × g pellet, 22% sedimented at 170,000 × g, and 12% was recovered in the soluble fraction (Table IV). This distribution closely parallels the distribution pattern of dolichol-phosphate mannose synthase, a marker for endoplasmic reticulum, while distribution profiles of the other markers were clearly different. To increase the resolution of the analysis, the 10,000 × g pellet was subjected to sucrose density gradient fractionation (17). Again, the fractionation profile of HT-T7-Gtt1p is nearly identical to the distribution of dolichol-phosphate mannose synthase, and distinct from distribution of other markers (Fig. 4).

Transcriptional Regulation of GTT1—The promoter region of GTT1 contains a xenobiotic response element (30), and an antioxidant response element (31) found in several mammalian GST genes. It also contains several copies of a stress response element (STRE; Refs. 32 and 33) and a post-diauxic shift element (PDS; Refs. 34 and 35), found in several S. cerevisiae genes, and a sequence recognized by yeast yAP1 transcriptional factor (36). To gain more insight about transcriptional regulation of GTT1, we fused its promoter to lacZ gene in centromeric plasmid pSEYC102 and determined β-galactosidase activity during growth of strain JC105. The activity increased dramatically at the end of the exponential phase (diiauxic shift) and remained high throughout the stationary phase (Fig. 5). A similar promoter activation profile was found in SSA3 gene (HSF70 homolog in S. cerevisiae). SSA3 promoter contains a sequence (PDS element) capable of activating transcription after glucose depletion in the medium (diiauxic shift) and during the stationary phase (34). The transcriptional regulation mediated by PDS is under control of RAS/cAMP pathway (32). Our results suggest that the PDS element in GTT1 promoter is functional, and that the GTT1 expression is up-regulated at the end of the exponential phase. This conclusion is supported by a recent report demonstrating the potential of DNA microarrays technology for genome-wide studies of transcriptional regulation in S. cerevisiae, which identified an open reading frame YIRO38c (GTT1) as transcriptionally up-regulated at the end of logarithmic growth and throughout the stationary phase (37). To determine the effect of different stress conditions and xenobiotics (such as GST substrates) on transcriptional regulation of GTT1, JC105 cells containing the reporter plasmid were precultured under selection (SD-uracil) and then diluted in YPD medium to A500 of 0.15. Cultures were grown to A500 of 1.3–1.5 and subjected to various stress conditions or chemicals for 1 h, and β-galactosidase activity was determined in cell lysates (Table V). Expression of β-ga-

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**Table II**

| Fraction                  | Protein | Total activity | Specific activity | Purification | Yield |
|---------------------------|---------|----------------|------------------|--------------|-------|
| Extract before induction  | ND      | 1             | 1                | ND           | ND    |
| Extract after induction   | 305.4   | 353.4         | 1.2              | 100          | 66    |
| Ni^2+ - agarose           | 7.2     | 234.1         | 32.5             | 27           | 66    |

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**Table III**

| Fraction                  | Protein | Total activity | Specific activity | Purification | Yield |
|---------------------------|---------|----------------|------------------|--------------|-------|
| Extract before induction  | ND      | 1             | 1                | ND           | ND    |
| Extract after induction   | 107.6   | 390.6         | 3.6              | 100          | 15    |
| Ni^2+ - agarose           | 1.6     | 60.3          | 37.2             | 10           | 15    |

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*ND, not determined.

a The GST activity in the extract before induction was not detectable.
Gtt1p and Gtt2p Function in Stationary Phase—To determine the phenotype resulting from disruptions of GTT1 and/or GTT2 genes, we constructed in vitro two deletion alleles by replacing most of the GTT1 gene with either TRP1 or URA3 nutritional markers. The GTT2 gene was replaced with URA3 marker. DNA fragments containing the disruption alleles were transformed into a haploid W303-1a strain, and the transformants were selected for tryptophan or uracil prototrophy. Since the transformants containing single disruptions gtt1::URA3 (strain JC100, gtt1::URA3 (strain JC101), or gtt2::URA3 (strain JC102) were viable, we have also disrupted GTT2 in strain JC100 to create a double disruptant strain, JC103. The growth rate of the strains W303-1a, JC100, JC101, and JC103 was found to be indistinguishable at all temperatures tested (15 °C-37 °C) on both rich medium (YPD) and synthetic medium (SD). In addition, the growth rate was not altered when glucose in the media was replaced with an alternative carbon source (galactose, raffinose, ethanol, glycerol, acetate) or when the wild type cells (W303–1a), as determined by plating on YPD plates. This difference is relatively small, but reproducible.

Since the promoter region of GTT1 contains STRE and PDS elements, and the transcription from the promoter is up-regulated after diauxic shift, Gtt1p may function in some aspect of differentiation into the stationary phase. One of the characteristics of S. cerevisiae cells in the stationary phase is a development of constitutive thermotolerance. We compared a survival ability of the W303-1a, JC100, JC102, and JC103 cells in the stationary phase after a heat shock at 52 °C for 5 and 10 min (Fig. 6). The JC100, JC102, and JC103 cells exhibited about 30% reduction in survival in comparison with the wild type cells (W303-1a), as determined by plating on YPD plates. This difference is relatively small, but reproducible. In addition, when grown at 39 °C, JC100, JC102, and JC103 strains reach the stationary phase at lower culture density than the wild type strain W303-1a (Fig. 7). This result was confirmed by determining the number of viable

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Since the promoter region of GTT1 contains STRE and PDS elements, and the transcription from the promoter is up-regulated after diauxic shift, Gtt1p may function in some aspect of differentiation into the stationary phase. One of the characteristics of S. cerevisiae cells in the stationary phase is a development of constitutive thermotolerance. We compared a survival ability of the W303-1a, JC100, JC102, and JC103 cells in the stationary phase after a heat shock at 52 °C for 5 and 10 min (Fig. 6). The JC100, JC102, and JC103 cells exhibited about 30% reduction in survival in comparison with the wild type cells (W303-1a), as determined by plating on YPD plates. This difference is relatively small, but reproducible. In addition, when grown at 39 °C, JC100, JC102, and JC103 strains reach the stationary phase at lower culture density than the wild type strain W303-1a (Fig. 7). This result was confirmed by determining the number of viable...
cells in the stationary cultures by plating on YPD media and growing the colonies at 30 °C. Stationary cultures (between 40 and 100 h) of W303–1a maintained 1.5–2.0 fold more viable cells than JC100, JC102, and JC103. Deletion of GTT1 and GTT2 does not seem to have an additive effect on the sensitivity to heat shock or the ability to grow at 39 °C (Figs. 6 and 7). Thus, Gtt1p and Gtt2p seem to have largely overlapping functions in stationary phase cells.
TABLE V
Stress induction of a GTT1-lacZ fusion gene
JC105 cells containing the reporter plasmid were grown in YPD medium to A_{600 \text{ nm}} of 1.3–1.5, subjected to various stress conditions or chemicals for 1 h, and $\beta$-galactosidase activity and the protein concentration was determined in the cell lysates as described under "Experimental Procedures." Values shown are the averages of at least three independent experiments, which agreed within 10%.

| Treatment       | Concentration | Specific activity |
|-----------------|---------------|------------------|
|                 | mM            | nmol/min/mg       |
| Control         | 300.0         | 1.6              |
| Heat shock      | 200.0         | 12.0             |
| pH 2.8          | 400.0         | 5.6              |
| CaCl$_2$        | 0.2           | 3.5              |
| MgCl$_2$        | 0.2           | 4.0              |
| NaCl            | 0.2           | 2.5              |
| CdCl$_2$        | 0.4           | 3.9              |
| Sorbitol        | 1.0           | 4.8              |
| Hydroquinone    | 0.25          | 5.2              |
| $\alpha$-Dinitrobenzene | 0.2 | 3.5 |
| CDNB            | 0.2           | 4.0              |
| $\text{H}_2\text{O}_2$ | 0.4 | 2.5 |
| $t$-Butyl $\text{H}_2\text{O}_2$ | 1.0 | 3.9 |
| pH 2.8          | 0.2           | 3.5              |
| Control         | 0.2           | 3.5              |
| Heat shock      | 0.2           | 4.0              |
| pH 2.8          | 0.4           | 2.5              |
| CaCl$_2$        | 1.0           | 3.9              |
| MgCl$_2$        | 0.2           | 3.5              |
| NaCl            | 0.2           | 4.0              |
| CdCl$_2$        | 0.4           | 2.5              |
| Sorbitol        | 1.0           | 3.9              |
| Hydroquinone    | 0.25          | 5.2              |

DISCUSSION
Stationary phase cells are stressed by a lack of nutrients and accumulation of toxic metabolites, and are differentiated in ways that allow to maintain their viability for extended periods without added nutrients. Some of the changes in the stationary phase cells resemble the changes in cells undergoing stress response, such as induction of heat-shock genes HSP26, HSP12, HSP92, HSP104, SSA3, and accumulation of trehalose (38). Induction of HSP104 and accumulation of trehalose are the main factors required for the stationary phase thermotolerance. Elliot et al. (39) reported that mutant strain, which is not able to synthesize trehalose and which is deleted for hsp104, is still slightly more thermoresistant in stationary phase than the wild-type cells in log phase. This suggests that some additional mechanisms are required for the cell thermoresistance, and our data indicate that at least some of these factors are Gtt1p and Gtt2p.

Elimination of lipophilic toxins from the cytosol is, after their conjugation with GSH, mediated by GS-X pumps, such as the human multidrug resistance-associated protein (MRP1; Refs. 40–42), the yeast cadmium factor protein (Ycf1p; Refs. 14–16, 43, and 44) and possibly the yeast Yor1p protein (45). Through the concerted actions of GSTs and the GS-X pumps, cells can confer a common structural determinant on toxins, increase their water solubility, and then eliminate them from the cytosol (1, 46). The Ycf1p localizes to the vacuole and is required for an increased resistance to the toxic effects of CDNB (14), a substrate of Gtt1p and Gtt2p, and a precursor of S-(2,4-dinitrophenyl)glutathione (DNP-GS). Since Gtt1p and Gtt2p operate in this detoxification pathway before Ycf1p, we expected them to be required for an increased resistance to CDNB as well. However, disruption of GTT1 and/or GTT2 genes (cells JC100, gtt1D, JC102, gtt2D, and JC103, gtt1D gtt2D) did not result in an increased sensitivity to CDNB. Since the S. cerevisiae genome does not contain another open reading frame even distantly similar to the homologous domain of GST genes, and the cell lysates of JC103 (gtt1D gtt2D) cells do not exhibit any detectable GST activity, the existence of additional S. cerevisiae gene(s) coding for protein with GST activity can be excluded. One reason for the CDNB resistance of the JC103 (gtt1D gtt2D) cells might be that CDNB is eliminated by at least one of the ABC transporters (9) and does not require conjugation with GSH (catalyzed by Gtt1p and Gtt2p). It is possible that Ycf1p is able to transport not only DNP-GS, but also CDNB. This would explain the observed sensitivity of ycf1D cells to CDNB (14). Alternatively, there could be a parallel, redundant, and Gtt1p- and/or Gtt2p-dependent pathway for CDNB elimination. In this case, Ycf1p would be the only ABC transporter capable of eliminating DNP-GS from the cytosol. In this scenario, CDNB would be converted to DNP-GS by Gtt1p and/or Gtt2p, and DNP-GS would be transported to the vacuole by Ycf1p. However, in ycf1D cells, DNP-GS would accumulate in the cytosol and cause the toxicity of CDNB. It is possible, that in the case...
Gtt1p and Gtt2p will provide important new information about other stress conditions (52, 53). Understanding the function of these proteins with the hypothesis that protein damage might be the same as heat shock proteins, and increase stability of other proteins. The microsomal GST also exhibits a GSH peroxidase activity, catalyzes reduction of lipid hydroperoxides to their corresponding alcohols, and thus plays a role in protection of membrane lipids against reactive oxygen species (48–50). However, we did not detect any GSH peroxidase activity associated with Gtt1p or Gtt2p (data not shown). Furthermore, JC100, JC102, and JC103 cells are not more sensitive to the oxidative stress caused by H2O2, butylhydroperoxide, cumene hydroperoxide, menadione, or diazoamino-1,2-cyclohexene (48). However, we did not detect any GSH peroxidase activity associated with Gtt1p or Gtt2p (data not shown). Furthermore, JC100, JC102, and JC103 cells are not more sensitive to the oxidative stress caused by H2O2, tert-butylhydroperoxide, cumene hydroperoxide, menadione, or diazoxide, butylhydroperoxide, cumene hydroperoxide, menadione, or diazoxide.

Both the induction of GTT1 expression after diauxic shift and the increased sensitivity to heat shock of stationary phase cells deleted for GTT1 and/or GTT2 demonstrate function of the corresponding protein products in stress response and in the elimination of toxic metabolites that accumulate during the stationary phase as well as under other stress conditions. This explanation is supported by recent findings that GSH, in addition to its role in protection against reactive oxygen species, may have another role in the elimination of toxic intermediates that arise during normal cellular metabolism (51). Alternatively, Gtt1p and Gtt2p may act in a similar fashion as heat shock proteins, and increase stability of other proteins under stress conditions. This explanation is in agreement with the hypothesis that protein damage might be the primary cause of the toxicity and lethality of heat stress and other stress conditions (52, 53). Understanding the function of Gtt1p and Gtt2p will provide important new information about physiologic processes and detoxification mechanisms in the stationary phase cells.

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