EUKARYOTIC CELL, Oct. 2006, p. 1748–1759 Vol. 5, No. 10
1535-9778/06/$08.00+0 doi:10.1128/EC.00216-06

A Peroxisomal Glutathione Transferase of *Saccharomyces cerevisiae* Is Functionally Related to Sulfur Amino Acid Metabolism▽

Lina Barreto,[1] Ana Garcerá,[1] Kristina Jansson,[2] Per Sunnerhagen,[2] and Enrique Herrero[1]*

Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, 25008 Lleida, Spain,[1] and Department of Cell and Molecular Biology, Lundberg Laboratory, Göteborg University, S-405 30 Göteborg, Sweden[2]

Received 11 July 2006/Accepted 8 August 2006

Saccharomyces cerevisiae **cells contain three omega-class glutathione transferases with glutaredoxin activity (Gto1, Gto2, and Gto3), in addition to two glutathione transferases (Gtt1 and Gtt2) not classifiable into standard classes. Gto1 is located at the peroxisomes, where it is targeted through a PTS1-type sequence, whereas Gto2 and Gto3 are in the cytosol. Among the *GTO* genes, *GTO2* shows the strongest induction of expression by agents such as diamide, 1-chloro-2,4-dinitrobenzene, *tert*-butyl hydroperoxide or cadmium, in a manner that is dependent on transcriptional factors Yap1 and/or Msn2/4. Diamide and 1-chloro-2,4-dinitrobenzene (causing depletion of reduced glutathione) also induce expression of *GTO1* over basal levels. Phenotypic analyses with single and multiple mutants in the *S. cerevisiae* glutathione transferase genes show that, in the absence of Gto1 and the two Gtt proteins, cells display increased sensitivity to cadmium. A *gto1*-null mutant also shows growth defects on oleic acid-based medium, which is indicative of abnormal peroxisomal functions, and altered expression of genes related to sulfur amino acid metabolism. As a consequence, growth of the *gto1* mutant is delayed in growth medium without lysine, serine, or threonine, and the mutant cells have low levels of reduced glutathione. The role of Gto1 at the *S. cerevisiae* peroxisomes could be related to the redox regulation of the Str3 cystathionine β-lyase protein. This protein is also located at the peroxisomes in *S. cerevisiae*, where it is involved in transulfuration of cysteine into homocysteine, and requires a conserved cysteine residue for its biological activity.**

Glutathione transferases (GSTs) conjugate xenobiotics or their metabolites to glutathione (GSH), followed by elimination of the conjugates from the cell through ATPase pumps (2, 34, 64). In addition, GSTs are also important in processes such as protection against oxidative stress, regulation of gene expression, or signal transduction (4, 34, 51, 64, 70). GSTs are divided into classes based on sequence, substrate specificity, or immunological properties. A majority of studies have been done with mammalian cytosolic GSTs, for which the division into classes was initially applied. However, GSTs also exist as membrane-associated (microsomal) forms, as well as in organelles such as mitochondria or peroxisomes (34, 46). Bacteria and eukaryotes such as fungi or parasites also contain GSTs, although these are structurally and immunologically different from mammalian or plant GSTs (17, 47, 64, 75).

Only a few fungal GST and GST-like proteins have been studied to some extent (47). The yeast *Saccharomyces cerevisiae* has two proteins with GST activity, the products of the genes *GTT1* and *GTT2* (11). Gtt1 is associated with the endoplasmic reticulum and could be involved in thermotolerance (11). Gtt1 and Gtt2 are functionally related to dithiol glutaredoxins Grx1 and Grx2 in defense against oxidants and other stresses (14). In addition to their thiol transferase activity, Grx1 and Grx2 are active as glutathione peroxidases and have activity against standard GST substrates (14, 15). Purified Gtt1 also displays activity as glutathione peroxidase against organic hydroperoxides (25). Overlapping functions therefore seem to exist between the Gtt1/Gtt2 and the Grx1/Grx2 proteins. *Schizosaccharomyces pombe* has three homologues of Gtt1/Gtt2 (named Gst1, Gst2, and Gst3) also with a function in defense against hydroperoxides (72). A role of *S. cerevisiae* Gtt1 and Gtt2 has been proposed in cadmium stress through the possible formation of Cd-GSH complexes (1). However, a *gtt1 gtt2* mutant is not hypersensitive to Cd (56), raising doubts on the function of the Gtt proteins in Cd detoxification. Intriguingly, the Gtt homologue Ure2, which is devoid of glutathione peroxidase and GST activity, participates in the detoxification of Cd and other heavy metals (56, 57). The relationship between Cd stress and GSH metabolism is nevertheless supported by two facts: (i) expression of the *GSH1* gene (coding for the first enzyme of the GSH biosynthetic route, γ-glutamylcysteine synthetase) is induced by Cd through a mechanism that is dependent on transcriptional regulators that modulate the sulfur amino acid biosynthetic route (19) and (ii) when yeast cells are exposed to Cd most of the assimilated sulfur is redirected to GSH synthesis (23).

Omega class GSTs diverge from other GST classes because they have low or no activity against standard GST substrates, whereas they are active as glutaredoxins/thiol oxidoreductases and dehydroascorbate reductases through a single cysteine residue at the active site (6, 80). Thus, omega GSTs are active as redox regulators of thiol groups, with GSH as a reductant. Omega GSTs or homologues for which enzyme activity has not yet been characterized are widespread in evolutionary divergent organisms from bacteria to mammals (25, 30, 60, 80). *S. cerevisiae* cells contain three omega class GSTs (Gto1, Gto2,

* Corresponding author. Mailing address: Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, Montserrat Roig 2, 25008 Lleida, Spain. Phone: (34) 973-702409. Fax: (34) 973-702426. E-mail: enric.herrero@cmb.udl.es.

▽ Published ahead of print on 25 August 2006.

TABLE 1. Strains used in this study

Strain	Relevant genotype	Comments
W303-1A	*MAT***a** *ura3-1 ade2-1 leu2,3-112 trp1-1 his3-11,15*	Wild type
W303-1B	*MAT*α *ura3-1 ade2-1 leu2,3-112 trp1-1 his3-11,15*	Wild type
MML535	*MAT***a** *gto1*::*natMX4*	Deletion of *GTO1* in W303-1A
MML536	*MAT*α *gto1*::*natMX4*	Deletion of *GTO1* in W303-1B
MML538	*MAT***a** *gto2*::*kanMX4*	Deletion of *GTO2* in W303-1A
MML540	*MAT*α *gto2*::*kanMX4*	Deletion of *GTO2* in W303-1B
MML542	*MAT***a** *gto3*::*natMX4*	Deletion of *GTO3* in W303-1A
MML546	*MAT*α *gto1*::*kanMX4*	Deletion of *GTO1* in W303-1B
MML572	*MAT***a** [pMM401(*GTO2-3HA*)]::*URA3*	Integration of pMM401 in W303-1A
MML587	*MAT***a** *gto1*::*kanMX4 gto3*::*natMX4*	From a cross, MML542 × MML546
MML590	*MAT*α *gto1*::*kanMX4 gto2*::*kanMX4 gto3*::*natMX4*	From a cross, MML540 × MML587
MML628	*MAT***a** *gtt1*::Ca*URA3*	Deletion of *GTT1* in W303-1A
MML629	*MAT***a** *gtt2*::*kanMX4*	Deletion of *GTT2* in W303-1A
MML630	*MAT***a** *gtt2*::*natMX4*	Deletion of *GTT2* in W303-1A
MML634	*MAT*α *gto1*::*natMX4 gtt1*::Ca*URA3*	From a cross, MML536 × MML628
MML636	*MAT*α *gto2*::*kanMX4 gtt1*::Ca*URA3*	From a cross, MML540 × MML628
MML661	*MAT***a** *gtt1*::Ca*URA3 gtt2*::*kanMX4*	From a cross, MML629 × MML634
MML666	*MAT***a** *gtt1*::Ca*URA3 gtt2*::*natMX4*	From a cross, MML630 × MML636
MML686	*MAT***a** *gto1*::*kanMX4 gto2*::*kanMX4 gto3*::*natMX4*	From a cross, MML540 × MML587
MML687	*MAT***a** *gto1*::*kanMX4 gto2*::*kanMX4 gtt1*::Ca*URA3 gtt2*::*natMX4*	From a cross, MML590 × MML666
MML716	*MAT***a** *gto1*::*kanMX4 gto2*::*kanMX4 gto3*::*LEU2 gtt1*::Ca*URA3 gtt2*::*natMX4*	Disruption of *GTO3* in MML687, using pMM550
MML826	*MAT***a** *str3*::*kanMX4*	Deletion of *STR3* in W303-1A
Wmsn2msn4	*MAT***a** *msn2*::*HIS3 msn4*::*TRP1*	From F. Estruch; deletion of *MSN2* and *MSN4* in W303-1A
Wyap1	*MAT***a** *yap1*::*kanMX4*	From F. Estruch; deletion of *YAP1* in W303-1A
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Wild type
Y03603	*MAT***a** *pex5*::*kanMX4*	From EUROSCARF[a]; deletion of *PEX5* in BY4741
Y04076	*MAT***a** *pex7*::*kanMX4*	From EUROSCARF; deletion of *PEX5* in BY4741

[a] EUROSCARF, European *Saccharomyces cerevisiae* Archive for Functional Analysis (University of Frankfurt; http://web.uni-frankfurt.de/fb15/mikro/euroscarf).

and Gto3) with an enzyme activity pattern similar to human omega GSTs hGTO1-1 and hGTO2-2 (25). Studies with purified Gto2 indicate that a single cysteine conserved in other omega GSTs is sufficient for its glutaredoxin activity, therefore indicating that this occurs through a monothiol mechanism of action (25). In the present study we demonstrated that Gto2 and Gto3 are located at the cytosol, whereas Gto1 is peroxisomal. We also analyzed the expression of the three genes in different stress conditions and carried out a phenotypic study of the mutants in relation to mutants lacking the *GTT* genes. These functional analyses point to a role of Gto1 in the metabolism of sulfur amino acids at the peroxisomes and propose a relationship between Gto1 and cystathionine β-lyase activity.

MATERIALS AND METHODS

Strains and growth conditions. The *S. cerevisiae* strains used in the present study are described in Table 1. The following rich media were used for cell growth: YPD (1% yeast extract, 2% peptone, 2% glucose), YPGly (1% yeast extract, 2% peptone, 3% glycerol), and YPOle (1% yeast extract, 2% peptone, 0.2% oleic acid, 0.02% Tween 40). Defined SC or SD media were also used, with the auxotrophic requirements for each strain (65); glucose at 2% or oleic acid (0.2%) plus Tween 40 (0.02%) was added as a carbon source. Two percent agar was added in solid media. Sulfur-free B medium (in some cases solidified with 1% agarose [10]) was used to analyze growth ability on different sulfur sources. Cultures were incubated at 30°C. Before cells were grown in oleic acid-based medium, they were preincubated in YPD medium (or in SC plus glucose in case of plasmid transformants) to a cell density of 3×10^7 cells/ml and then resuspended at 0.3 to 0.5×10^7 cells/ml in YPOle (or SC plus oleic acid) medium and incubated in these conditions for the indicated times.

Plasmids. Plasmids pMM399 and pMM446 contain C-terminal green fluorescent protein (GFP) fusions to *GTO2* and *GTO3*, respectively, under the control of the *MET25* promoter in the pUG35 vector (from W. H. Hegemann, Institute of Microbiology, Dusseldorf, Germany). The complete *GTO2* and *GTO3* coding sequences (PCR-amplified) were cloned between the XbaI-EcoRI (for pMM399) or BamHI-ClaI (for pMM446) vector sites. Integrative plasmid pMM401 is a YIplac211 (29) derivative with *GTO2* under its own promoter, tagged at the C end with three hemagglutinin (HA) epitopes in tandem. This plasmid was used to construct strain MML572, after EcoRV-mediated linearization of pMM401, transformation of wild-type cells, and integration at the chromosomal *URA3* site. pMM440 is a centromeric *URA3* plasmid that carries the complete coding sequence of *GTO1* under the control of the $tetO_7$ promoter from pCM189 (26), with an N-terminal fusion of the coding sequence to the GFP tag. Plasmid pMM736 is based in YCplac211 (29) and contains the *GTO1* gene expressed under its own promoter and TAP tagged at its N terminus; the TAP tag was PCR amplified from plasmid pBS1761 (54). To generate the internal disruption of *GTO3* in strain MML716, plasmid pMM550 was constructed. This plasmid is based on YIplac211 and contains the *GTO3* sequence under its own promoter, C-end tagged with 3-HA and disrupted at the internal XbaI site of *GTO3* with a *LEU2* gene plus promoter cassette amplified from YIplac128 (29). Then, a DNA fragment that contains the *LEU2* marker cassette flanked at both ends by 250 bp of the *GTO3* coding sequence was PCR amplified from pMM550 for disruption of the wild-type *GTO3* gene. Plasmid pMM756 contains the *STR3* open reading frame expressed from the $tetO_2$ promoter, cloned between the BamHI and NotI sites of pCM188 (26). The following plasmids derive from pMM756 and express point mutant derivatives of *STR3*: pMM758 (Cys353Ser), pMM760 (Cys362Ser), and pMM762 (Cys387Ser).

Genetic methods. Standard protocols were used for DNA manipulations and cell transformations. Point mutants were constructed with the ExSite method (79), using oligonucleotides for PCR amplification that introduced the desired mutation and proximally inserted a restriction enzyme site that did not alter the translation product. Mutations were confirmed by DNA sequencing. Single null mutants were obtained by using the short-flanking homology approach after PCR amplification of the *kanMX4* (76), the *natMX4* (32), or the *CaURA3MX* (31) cassettes. Disruptions were confirmed by PCR analysis. Multiple mutants were obtained by crossing the desired parental mutant strains, followed by diploid sporulation, tetrad analysis, and selection of the desired mutant combinations (65).

Determination of chemical sensitivities. Bioscreen automated analysis of the effect of different inhibitors on yeast cell growth was done with cells cultured in

supplemented SD liquid medium at 30°C, using procedures described previously (78). A range of concentrations were tested beforehand for each chemical to determine the concentration value decreasing the exponential growth rate in wild-type cells by 20%. This value was used in further experiments. The results were scored during 48 h of growth at 30°C at high intensity shaking. The time interval between optical density (600 nm) readings was 20 min.

Microarray analysis. About 3×10^8 wild-type or mutant cells growing exponentially in YPD medium were collected, and total RNA was extracted by using the RiboPure-Yeast RNA purification kit (Ambion). For each array hybridization experiment, labeled cDNA was synthesized from a wild-type or mutant sample (30 μg of total RNA) by using the CyScribe postlabeling kit in the presence of Cy3-dUTP or Cy5-dUTP (GE Healthcare). Dyes were swapped for wild-type and mutant cells in independent experiments to avoid dye-specific bias. Fluorescence-labeled cDNAs from both strains were combined and hybridized to yeast genomic microarrays. The characteristics of these and the prehybridization, hybridization, and washing conditions have been described elsewhere (74). Hybridized arrays were scanned with an Axon 4100A scanner (Axon Instruments, Inc.), and fluorescence ratio measurements were analyzed by using the GenePix Pro 5.0 software (Axon Instruments, Inc.). The data from three independent experiments in which both strains were labeled in parallel were used to calculate the median of the ratio for each gene. A given gene was considered to be differentially expressed between both strains when the wild-type/mutant ratio was higher than 2.0 or lower than 0.5 in at least two of the three experiments, and the dyes were swapped in the considered experiments.

In silico analysis. A search for promoter regulatory sequences was made at the regulatory sequence analysis tools site (http://rsat.scmbb.ulb.ac.be/rsat) (71). The MITOPROT program (13) was used to predict mitochondrial targeting sequences. Multiple sequence alignments were done with CLUSTAL W (68), using the European Bioinformatics Institute tools (www.ebi.ac.uk).

Miscellaneous methods. Mitochondria were purified and subfractionated (18) from exponential yeast cultures in YPGly medium at a cell concentration of 2×10^7 cells per ml. Western blot analyses were done as described previously (59), with 12CA5 anti-HA MAb antibody (Roche Diagnostics) at a 1:5,000 dilution and anti-lipoic acid antibody at 1:50,000 dilution. In Northern blot studies, electrophoresis of RNA, probe labeling with digoxigenin, hybridization, and signal detection were carried out as previously described (26). Signals were quantified by using the Lumi-Imager equipment (Roche Diagnostics) software. Gene probes were generated from genomic DNA by PCR, using oligonucleotides designed to amplify internal open reading frame regions. Microscopic localization of GFP-labeled proteins in living cells was done by standard techniques (66). Total and reduced glutathione was measured as described previously (41). Cell volumes were determined with a Coulter Z2 particle analyzer.

RESULTS

Gto1 is located at peroxisomes, whereas Gto2 and Gto3 are cytosolic. In order to determine the cellular location of the yeast Gto proteins, we constructed GFP-fused versions of them. Gto1 has a C-terminal sequence compatible with a peroxisomal location. Most peroxisomal proteins are imported into the organelle by one of two alternative pathways, one that recognizes a PTS1 sequence at the protein C terminus and another one that recognizes an internal PTS2 sequence (36, 55). The C-terminal SRL amino acid sequence of Gto1 is one of the possible PTS1 variants in yeast cells (44, 73). To confirm the peroxisomal location of Gto1 microscopically, the GFP tag was added at the N terminus of the protein, and this was expressed under the control of the doxycycline-regulatable *tet* promoter. For Gto2 and Gto3 the tag was added at the C terminus and the construct expressed from the *MET25* promoter. Gto1 showed a punctate distribution in cells grown on oleic acid, which was not observed in glucose-grown cells (Fig. 1A). This pattern is characteristic of peroxisomal proteins (21). To confirm it, we studied the microscopic pattern of Gto1 in a Δ*pex5* mutant (deficient in PTS1-mediated import) and a Δ*pex7* mutant (deficient in PTS2-mediated import) (21, 73). The punctate location of Gto1 in oleic acid-grown cells was lost in the Δ*pex5* mutant, but not in Δ*pex7* cells (Fig. 1A). This therefore supported a PTS1-mediated import pathway for Gto1. The punctate pattern was still observed when gene expression was reduced by adding intermediate doxycycline concentrations (50 to 100 ng/ml) to the growth medium (not shown). These observations confirm previous studies based on in silico detection of yeast peroxisomal proteins (28) and also the experimental detection in a high-throughput screen of Gto1 in peroxisomes (82).

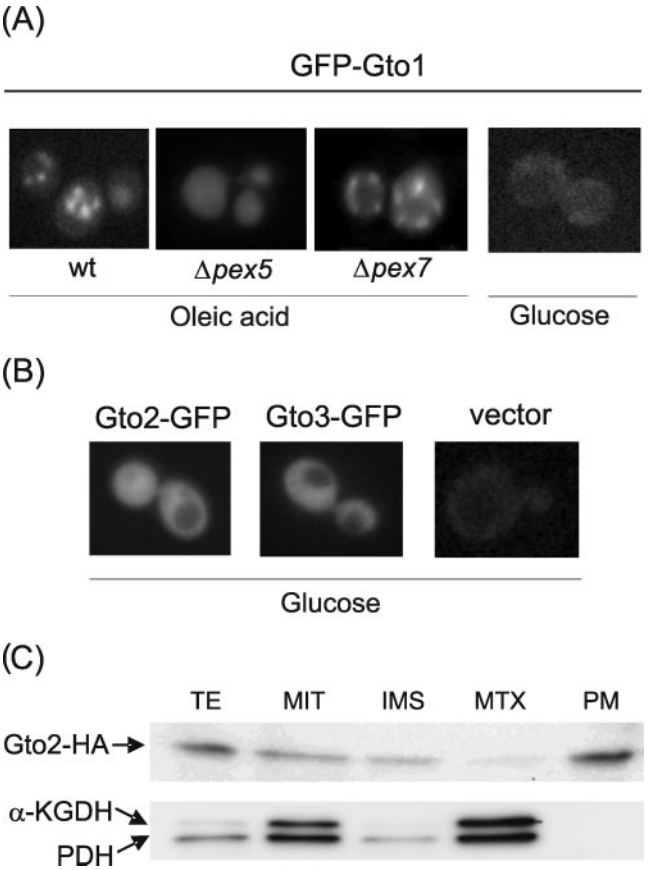

FIG. 1. Cellular localization of the Gto proteins. (A and B) Wild-type BY4741 cells or the respective Δ*pex5* (strain Y03603) and Δ*pex7* (strain Y04076) mutants transformed with the empty vector pUG35 or with the plasmids carrying the GFP-tagged *GTO* constructions (pMM440, pMM399, and pMM446, respectively, for *GTO1*, *GTO2*, and *GTO3*) were grown in oleic acid (YPOle) medium for 16 h at 30°C or in glucose (YPD) medium in exponential conditions and then observed by fluorescence microscopy. (C) MM572 cells (carrying a chromosomally integrated version of *GTO2-3HA*) were grown in YPD medium until late exponential phase and then diluted 1:100 in YPGly medium and cultured at 30°C until a concentration of 2.5×10^7 cells/ml before cellular fractionation was achieved. The resulting fractions were analyzed by Western blotting, using anti-HA antibodies to detect Gto2 and anti-lipoic acid antibodies to detect the mitochondrial markers pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (α-KGDH). Six micrograms of protein were loaded in each lane for total cell extracts (TE) and postmitochondrial supernatant (PM) fractions, and 3 μg was loaded for the mitochondrial (MIT), intermembrane space (IMS), and matrix (MTX) fractions.

We analyzed whether Gto homologues in other fungi also carried PTS1 motifs at their C termini. None of the Gto homologues in *Kluyveromyces lactis* (Swiss-Prot accession number

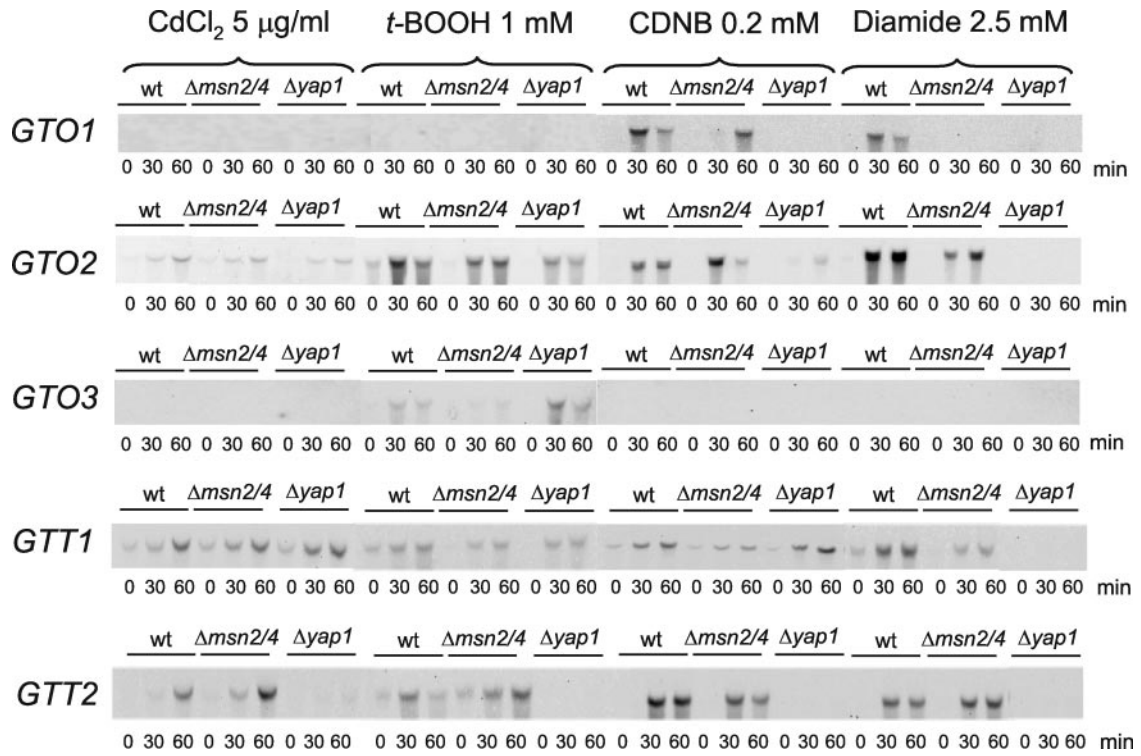

FIG. 2. Northern blot analysis of the expression of the *GTO1*, *GTO2*, *GTO3*, *GTT1*, and *GTT2* genes after addition of different agents. Cells from a wild-type (W303-1A) or the respective isogenic Δ*yap1* (Wyap1) or Δ*msn2 msn4* (Wmsn2msn4) strains were grown exponentially at 30°C in YPD medium, except in the cadmium experiments, in which cells were grown in SC medium. When cultures reached a concentration of 1.5×10^7 cells per ml, the agent was added at the indicated concentration (time zero), and samples were obtained at the indicated times for expression analysis. A total of 25 μg of total RNA was tested run per lane.

Q6CKB2), *Torulopsis glabrata* (Q6FTJ1), *Candida albicans* (Q5A953), *Yarrowia lipolytica* (Q6CDX7), *Debaryomyces hansenii* (Q6BTR1 and Q6BTR2), *Cryptococcus neoformans* (Q55K61 and Q55K65), *Schizosaccharomyces pombe* (O94524), *Neurospora crassa* (Q7RWR2), and *Aspergillus nidulans* (Q5BBA6 and Q5B0U9) contained PTS1 motifs. Only *Saccharomyces paradoxus* open reading frame c287_8925 (*Saccharomyces* Genome Database, http://db.yeastgenome.org/fungi), which displays a very strong homology with Gto1, has a PKL sequence at C terminus that is compatible with a PTS1 motif (44). None of these proteins contained N-terminal putative PTS2 sequences. Although we cannot totally discard the possibility that other motifs different from PTS1 or PTS2 could direct some of the Gto1 homologues to peroxisomes, our analyses strongly suggest that a peroxisomal location is specific for *S. cerevisiae* Gto1 and homologues from closely related yeast species.

Gto2 and Gto3 showed a homogeneous cytosolic distribution with different carbon sources (see Fig. 1B for glucose-grown cells). In silico analysis predicted a mitochondrial location for Gto2. To clarify this apparent contradiction, subfractionation studies were made with glycerol-grown cells carrying a C-terminal HA-tagged version of Gto2 expressed under its own promoter. Most of the Gto2 protein associated with the postmitochondrial supernatant (Fig. 1C), confirming the microscopic studies. A minor fraction appeared associated with the mitochondrial intermembrane space (Fig. 1C). This was ca. 3% of the total cell Gto2 protein, taking into consideration the cell protein equivalents in each of the fractions run in Fig. 1C. We conclude that the three Gto proteins constitute a family, one of whose members is peroxisomal, whereas the other two are located mostly if not totally in the cytosol.

***GTO* gene expression responds to oxidant conditions.** *S. cerevisiae* cells respond to oxidative stress caused by external agents by altering the expression of a large number of genes (27). A specific oxidative stress response is mediated by the Yap1 transcription factor, which recognizes and activates the YRE promoter element (TT/GACTAA) (35, 69). Gene induction by oxidants can also be part of the general stress response that is mediated by the Msn2 and Msn4 transcription factors recognizing promoter STRE elements (CCCCT) (22). Sequence analysis of the *GTO* promoters revealed the presence of an YRE site at position −170 in the *GTO1* promoter and at position −179 in the *GTO2* promoter; no canonical YRE sites exist in the *GTO3* promoter. All three *GTO* genes contain STRE sites in their promoters, at positions −241 in *GTO1*, −144 and −117 in *GTO2*, and at positions −701 and −330 in *GTO3*. This pointed to the possibility that *GTO* gene expression was induced in oxidative conditions. We therefore studied such expression (and its dependence on the Yap1 and Msn2/4 factors) in response to the oxidants *tert*-butyl hydroperoxide (*t*-BOOH) and diamide, the latter specifically causing the oxidation of sulfhydryl groups. The study was extended to the standard GST substrate 1-chloro-2,4-dinitrobenzene (CDNB) and also to cadmium. It has been proposed (14) that CDNB causes oxidative stress in yeast cells by deriving the GSH molecules into GSH-CDNB complexes due to the cellular GST

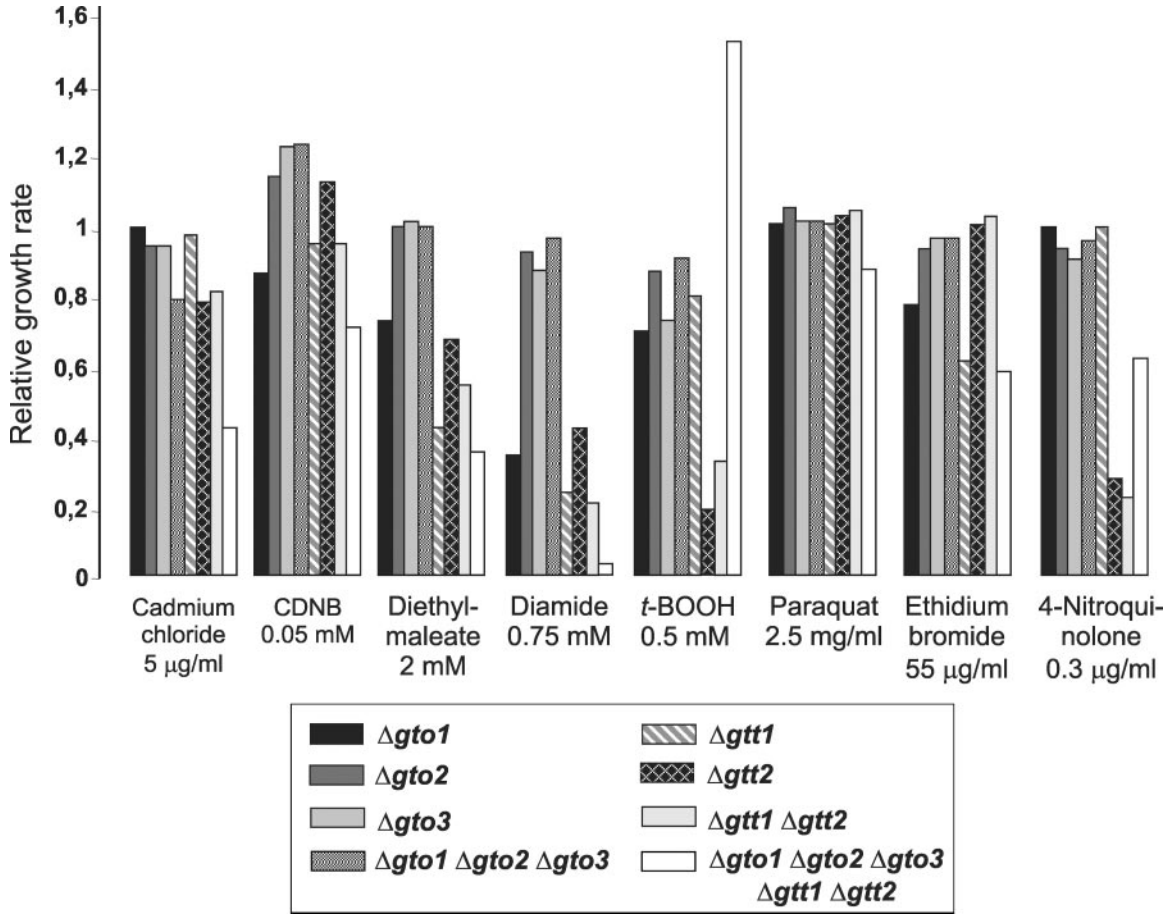

FIG. 3. Effect of different chemical agents on growth rate of *S. cerevisiae* wild-type cells (W303-1A) and *gtt* and *gto* mutant derivatives. The following mutant strains were studied: MML535 (Δ*gto1*), MML538 (Δ*gto2*), MML542 (Δ*gto3*), MML686 (Δ*gto1* Δ*gto2* Δ*gto3*), MML628 (Δ*gtt1*), MML629 (Δ*gtt2*), MMO661 (Δ*gtt1* Δ*gtt2*),and MML716 (Δ*gto1* Δ*gto2* Δ*gto3* Δ*gtt1* Δ*gtt2*). Conditions for growth were as described previously (80), and the respective agents were used at the indicated concentrations. Growth was recorded by measuring the optical density (600 nm) at 20-min intervals. Bars represent the ratios between the exponential growth rates of treated and untreated mutant cultures, and the values were normalized by dividing them by the corresponding ratio of the wild-type strain. The values of two independent experiments were averaged.

activity. Cd causes cell toxicity, at least in part, by provoking oxidative stress and lipid peroxidation (3, 9). Expression of the other two GST genes, *GTT1* and *GTT2*, was studied in parallel, since their promoters contain YRE elements (at −165 in *GTT1* and −98 in GTT2) and, in addition, the *GTT1* promoter has three STRE sites at positions −241, −211, and −114.

The basal expression of the three *GTO* genes in glucose-grown cells was almost under detection levels by Northern analysis (Fig. 2), although the corresponding HA-tagged Gto proteins expressed under their own promoters produced a significant signal in Western analyses (results not shown). Transcriptional response to the tested oxidants showed a complex pattern (Fig. 2). Among the three *GTO* genes, *GTO2* displayed the more intense response, since the four tested agents caused induction of transcript levels. This was abolished in a Δ*yap1* mutant in the case of diamide and CDNB and partially diminished in the case of *t*-BOOH and Cd. Msn2/4 seemed to play a more limited role in *GTO2* induction by the four agents, although transcript levels and/or the duration of the response were lower in the Δ*msn2* Δ*msn4* double mutant than in wild-type cells. The fact that the induction of *GTO2* transcription by Cd or *t*-BOOH was not totally eliminated in Δ*yap1* or Δ*msn2* Δ*msn4* mutants points to the participation of other factors, such as Skn7 (69). Only diamide and CDNB increased *GTO1* expression over basal levels, the induction being dependent on Yap1 and Msn2/4. *GTO3* expression was upregulated only by *t*-BOOH in an Msn2/4-dependent manner (Fig. 2). In summary, the dependence of *GTO* induction on the transcriptional activators Yap1 and Msn2/4 correlated with the presence of the respective binding sites at the promoters.

GTT1 was moderately induced by the four agents. Although its promoter contains putative STRE and YRE sites, only in the case of diamide was a dependence on Yap1 observed (Fig. 2). This leaves open the possible participation of other transcriptional factors in such activation. *GTT2* expression was also induced by the four tested agents, the induction in all cases being dependent on Yap1 but not on Msn2/4 (Fig. 2). This is in accordance with the presence of YRE sites and the absence of STRE elements in the *GTT2* promoter.

Mutants in *S. cerevisiae* genes coding for GSTs display several phenotypic defects. Induction of *GTO* and *GTT* gene expression by agents that cause oxidative stress pointed to the

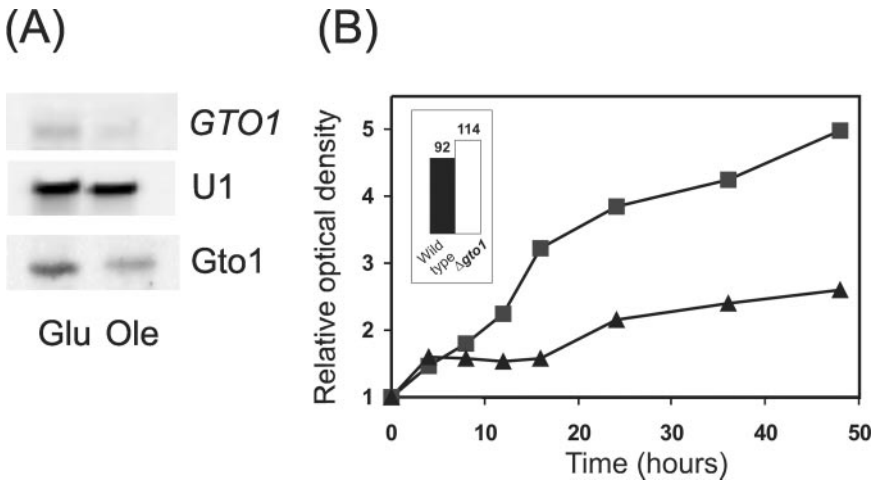

FIG. 4. (A) Expression of *GTO1* in glucose- and oleic acid-grown cells. Wild-type W303-1A cells transformed with pMM736 (expressing *GTO1* under its own promoter with a N-terminal TAP tag-expressing sequence) were pregrown in SC medium plus glucose at 30°C to about 4×10^7 cells/ml and then were diluted 10 times, and half of the culture was maintained in SC plus glucose, whereas the other half was shifted to SC plus oleic acid medium. Samples were obtained after 16 h at 30°C for Northern (upper two panels, U1 mRNA as a loading control) or Western (lower panel, 20 μg of total cell protein per lane) blot analysis. (B) The growth of wild-type W303-1A cells (■) and Δ*gto1* mutant cells (strain MML535 [▲]) in YPOle medium at 30°C was recorded. Cells were pregrown in YPD medium to about 3×10^7 cells/ml and diluted 10 times in YPOle medium (time zero). The optical density (600 nm) was measured at the indicated times and made relative to the unit value at time zero for the respective strain. The inset box shows the doubling times (in minutes) of the wild-type and mutant strains during exponential growth in YPD medium at 30°C.

importance of yeast GSTs in protection against oxidants. To confirm this, we carried out a high-resolution quantitative phenotypic analysis (78) using single and multiple *gto* and *gtt* mutants exposed to oxidants and other toxic agents (Fig. 3). Absence of one of the *GTT* genes or both together caused hypersensitivity to oxidants (diethylmaleate, diamide, *t*-BOOH) and, in the case of *GTT2*, also to the DNA-damaging agent 4-nitroquinolone. The results are in accordance with the proposed antioxidant role of Gtt1/Gtt2 (14) and suggest that Gtt2 activity is required to detoxify 4-nitroquinolone in yeast cells. Among the three *GTO* genes, the absence of *GTO1* caused significant sensitivity to diamide and more modest sensitivity to diethylmaleate and *t*-BOOH compared to wild-type cells (Fig. 3). In addition, a *gto1 gto2 gto3* mutant that also lacked the two *GTT* genes was hypersensitive to Cd. This pointed to some additive effect between Gto and Gtt functions in Cd detoxification. Surprisingly, the quintuple mutant was hyper-resistant to *t*-BOOH compared to any of the other mutant strains tested and also to wild-type cells (Fig. 3). The lack of the three Gto proteins also ameliorated the hypersensitivity of the *gtt2* and *gtt1 gtt2* mutants to 4-nitroquinolone.

Nonautomated measurements over a wider range of Cd concentrations confirmed the previous observations (not shown). Since the quintuple mutant *gtt1 gtt2 gto1 gto2 gto3* mutant did not display increased sensitivity to Cd over the quadruple mutant carrying the wild-type *GTO3* gene, this indicated that the latter was not important in protection against Cd effects. We then determined the sensitivity of double *gtt1 gtt2* mutants that additionally carried a single null mutation in each of the *GTO* genes. Only the absence of *GTO1* in a *gtt1 gtt2* background caused hypersensitivity to levels proximal to the quintuple mutant (not shown). That is, the Gto1 function, but not that of the

TABLE 2. Genes upregulated or downregulated (at least twofold) in the Δ*gto1* mutant relative to the wild-type strain[a]

Gene	Function	Δ*gto1*/wild-type ratio	Presence (+) or absence (−) of:	
			Met4/Met28/Cfb1element	Met4/Met31/Met32element
CHA1	Serine/threonine dehydratase	3.74	−	−
CIT2	Citrate synthase (peroxisomal)	2.21	−	+
AVO2	Unknown	2.15	−	−
MET16	3′-Phospho-5′-adenylylsulfate kinase	0.499	+	−
SAM3	*S*-Adenosyl-l-methionine permease	0.493	−	+
MUP1	High-affinity methionine permease	0.472	−	+
MET5	Sulfite reductase	0.471	+	−
MET17	Homocysteine synthase	0.450	+	+
SER3	3-Phosphoglycerate dehydrogenase	0.372	−	+
MET14	5′-Adenylylsulfate kinase	0.320	+	−
GAP1	General amino acid permease	0.186	−	+

[a] DNA microarray analyses were carried out after RNA extraction from exponentially growing cultures in YPD medium at 30°C. The presence or absence of promoter elements recognized by the Met4/Met28/Cbf1 (core sequence TCACGTG) and Met4/Met30/Met31 (core sequence CTGTGG) in the respective gene promoters is indicated. See also Fig. 5.

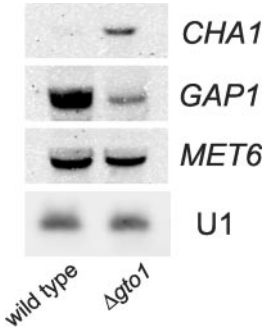

FIG. 5. Northern blot analysis of the expression of the indicated genes in Δ*gto1* and wild-type cells. Samples were obtained under the conditions described in Table 2. The small nuclear U1 RNA served as a loading control (25 μg of total RNA per lane).

other two Gto proteins, protects *S. cerevisiae* cells against Cd toxicity in cooperation with the Gtt1/Gtt2 functions.

***S. cerevisiae* cells lacking GTO1 are defective for growth on oleic acid medium.** The *GTO1* promoter lacks oleate response elements that are responsible for the induction of expression of many peroxisomal genes in *S. cerevisiae* cells growing on oleic acid (28). In accordance with this, *GTO1* expression was not induced when cells were shifted from glucose-based medium to oleic acid-based medium (Fig. 4). The relative levels of Gto1 protein were even lower in oleic acid than in glucose medium (Fig. 4A). However, the growth of *gto1* mutant cells was compromised when transferred from glucose medium to oleic acid medium compared to wild-type cells (Fig. 4). This contrasts with the situation when wild-type or mutant cells are exponentially growing in glucose-based rich medium without further additions, where differences in growth rates are minor (Fig. 4B, inset). Therefore, some Gto1 function is required for peroxisome-dependent growth of yeast cells using oleic acid as an energy source.

A *gto1* mutant is affected in the metabolism of sulfur amino acids. The results on Cd sensitivity indicated that the Gto1 protein plays some role not only when cells grow on oleic acid-based medium but also during fermentative growth on glucose. To address the role of Gto1 in these conditions, we used DNA microarrays to analyze the transcriptome of a Δ*gto1* mutant growing exponentially in YPD medium. Only a few significant (at least twofold) differences were observed in gene expression relative to wild-type cells (Table 2). Northern analysis of an upregulated (*CHA1*) and a downregulated gene (*GAP1*) confirmed the DNA microarray results for these genes (Fig. 5). A control gene (*MET6*) involved in the same meta-

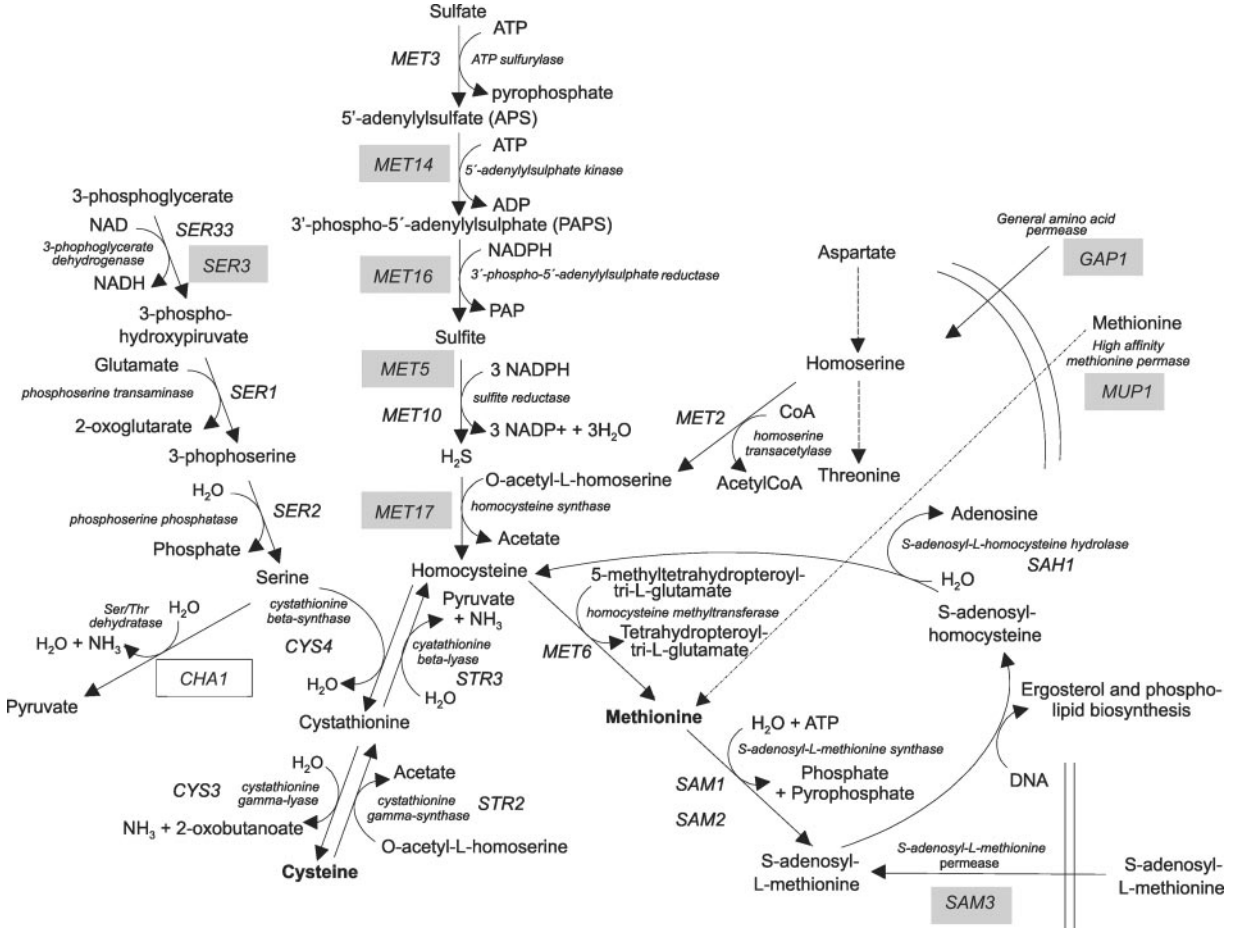

FIG. 6. Biochemical steps involved in the sulfur assimilation and biosynthesis of cysteine and methionine in *S. cerevisiae* and genes coding for the respective proteins. Genes boxed in white and gray correspond to upregulated and downregulated genes, respectively, in the Δ*gto1* mutant.

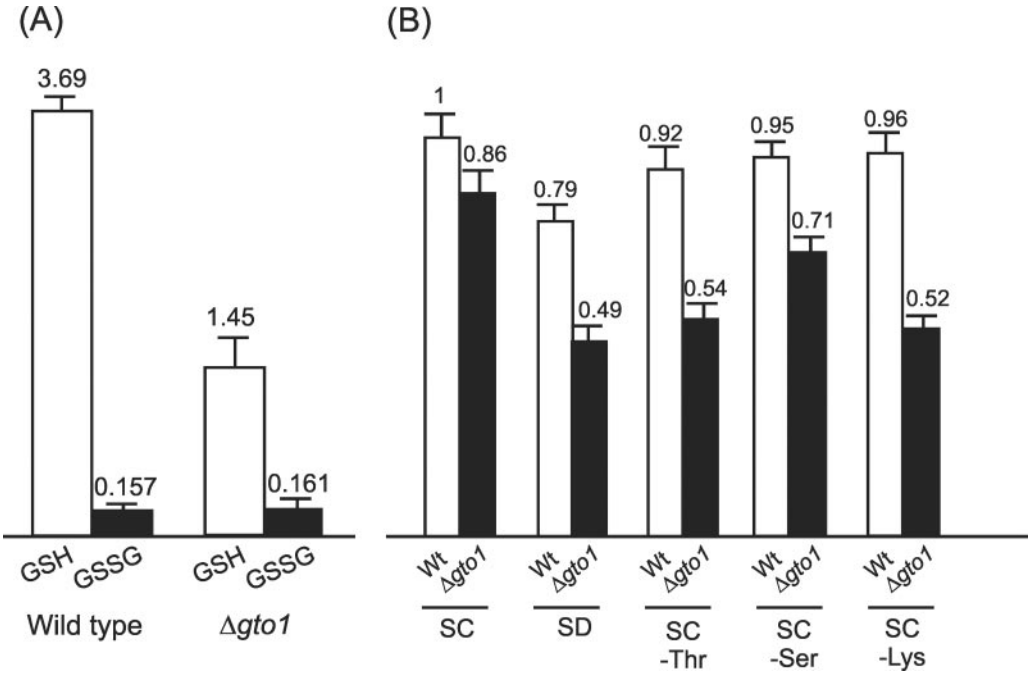

FIG. 7. The absence of *GTO1* causes an imbalance in the synthesis of reduced glutathione and a number of amino acids. (A) Intracellular millimolar concentrations of reduced (GSH) and oxidized (GSSG) glutathione in wild-type (W303-1A) and Δ*gto1* (MML535) cells. Absolute levels of both glutathione forms were determined in exponentially growing cells in YPD medium at 30°C. The intracellular glutathione concentration was calculated after the cell volumes were measured. Values are the means of three independent experiments (the standard deviation is indicated). (B) Growth rate of exponentially growing cultures at 30°C of wild-type (W303-1A [□]) and Δ*gto1* (MML535 [■]) cells in SC and SD medium and in SC medium without the individual amino acids indicated. Values were determined relative to wild-type cells in SC medium, which have a growth rate of 0.62 doublings per h.

bolic process as *CHA1* and *GAP1* (see below) and which did not show significant differences in the DNA microarray experiments also did not display significant differences in the Northern analysis (Fig. 5).

Most of the genes whose expression was altered in the mutant are involved in the metabolism of sulfur amino acids (Fig. 6) (67). Thus, upregulated *CHA1* participates in removing L-serine as a precursor of cysteine by converting the former to pyruvate. In contrast, downregulated *SER3* is required for the conversion of 3-phosphoglycerate to L-serine. Four of the downregulated genes (*MET14*, *MET16*, *MET5*, and *MET17*) participate in the sulfate assimilation pathway leading to homocysteine, which is the last common intermediate for the synthesis of cysteine and methionine. Finally, *GAP1*, *SAM3*, and *MUP1* are amino acid transporters, the latter two being specifically involved in the uptake of sulfur amino acids. Altogether, this pointed to a relationship between Gto1 and the sulfur amino acids biosynthetic pathway, and it could be hypothesized that the intracellular levels of sulfur amino acids would be reduced in the *gto1* mutant (Fig. 6). L-Cysteine is one of the three amino acids of the GSH molecule, and modification of intracellular cysteine concentration influences the GSH pool in the *S. cerevisiae* cell (19, 23). We therefore measured the levels of oxidized (GSSG) and reduced (GSH) glutathione in wild-type and Δ*gto1* cells. The mutant had significantly reduced levels of GSH, whereas the GSSH pool was not affected (Fig. 7A).

A Δ*gto1* mutant shows defective growth in SD minimal medium supplemented with the auxotrophic requirements compared to wild-type cells (Fig. 7B). Such a defect is not observed in SC defined medium supplemented with the 20 amino acids. In order to determine the amino acid deficiencies that provoked the slow growth rate of the mutant, growth in SC medium separately depleted of each of the amino acids was tested. The absence of serine caused a moderate growth defect in the mutant, whereas the absence of threonine or lysine caused a larger defect that was similar to that observed in SD medium (Fig. 7B). Therefore, the Δ*gto1* mutant has an imbalance in the biosynthesis of these three amino acids. The absence of cysteine or methionine in the growth medium did not lead to slow growth (not shown).

Transulfuration requires a peroxisomal function in *S. cerevisiae*. The relationship between Gto1 and the metabolism of sulfur amino acids suggested a role of the peroxisome in this metabolic pathway. In fact, the *S. cerevisiae* enzyme cystathionine β-lyase (the product of the *STR3* gene) contains a PTS1 sequence at the C end and has been experimentally detected at the peroxisome (61, 82). Other enzymes of the sulfur amino acid biosynthetic pathway do not display PTS motifs nor have been detected at the peroxisome. Str3 participates in the transulfuration pathway from cysteine to homocysteine (67), a process that occurs in bacteria, fungi, and plants but not in animals (37), by converting the intermediate cystathionine into homocysteine. In the absence of Str3 yeast cells are unable to grow on cysteine or cystathionine as the sole sulfur source (33). We reasoned that Gto1 could regulate the activity of Str3 through its redox modulator properties. Str3 has 10 cysteine residues and 3 of them (located at the C-terminal half of the molecule,

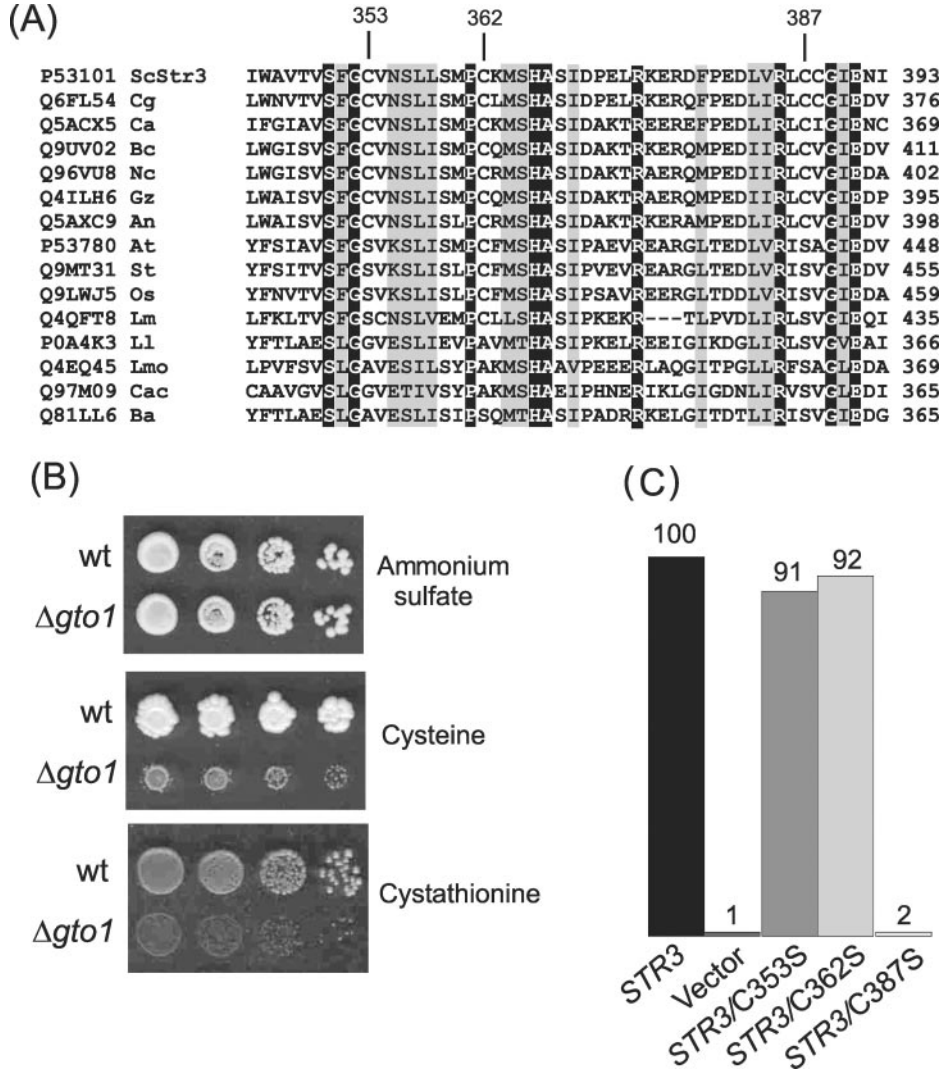

FIG. 8. Functional relationship between Gto1 and Str3 cystathione β-lyase. (A) CLUSTAL W multiple alignment of a region of cystathionine β-lyase molecules from diverse organisms that included three conserved cysteine residues (position marked for the residues in *S. cerevisiae* Str3). The abbreviations for the organisms were as follows: Sc, *Saccharomyces cerevisiae*; Cg, *Candida glabrata*; Ca, *Candida albicans*; Bc, *Botrytis cinerea*; Nc, *Neurospora crassa*; Gb, *Gibberella zeae*; An, *Aspergillus nidulans*; At, *Arabidopsis thaliana*; St, *Solanum tuberosum*; Os, *Oryza sativa*; Lm, *Leishmania major*; Ll, *Lactococcus lactis*; Lmo, *Listeria monocytogenes*; Cac, *Clostridium acetobutylicum*; Ba, *Bacillus anthracis*. (B) Growth of wild-type (W303-1A) and Δ*gto1* (MML535) cells on plates containing medium B, with glucose and ammonium sulfate, cysteine, or cystathionine as the sulfur source, after 3 days (for ammonium sulfate and cysteine) or 5 days (for cystathionine) at 30°C. (C) Mutant *str3* cells (strain MML826) transformed with plasmid pMM756 (carrying wild-type *STR3* gene), pCM188 (void vector), pMM758 (*STR3* with the C353S mutation), pMM760 (*STR3* with the C362S mutation), or pMM762 (*STR3* with the C387S mutation) were grown exponentially in SC medium, centrifuged and resuspended in B medium with cysteine at a concentration of 3×10^6 cells/ml. Cultures were incubated at 30°C and growth (optical density at 600 nm) was recorded 24 h later. Values are expressed relative to the *stre3* mutant transformed with the plasmid with wild-type *STR3*.

at positions 353, 362, and 387) are conserved in other fungal cystathionine β-lyase molecules (Fig. 8A), which points to their functional importance. To test the hypothesis that Str3 is functionally defective in the absence of Gto1, we studied the ability of a Δ*gto1* mutant to grow on cysteine or cystathionine as the sole sulfur source. In fact, compared to wild-type cells, the mutant shows growth deficiency on cysteine or cystathionine, with the deficiency being more intense in the latter (Fig. 8B). Cysteine at high concentrations is toxic to yeast cells (42). However, increasing the cysteine concentration up to tenfold over the standard concentration in medium B did not caused higher toxicity in the Δ*gto1* mutant than in wild-type cells (data not shown). Therefore, the growth deficiency in the mutant cannot be attributed to increased toxicity of cysteine (and probably cystathionine) on the mutant but to a defect in conversion of cysteine or cystathionine into homocysteine.

The three-dimensional structure of cystathionine β-lyase from *Arabidopsis thaliana* has been determined, and no cysteine residue is present at the enzyme binding site (7). However, the thiol-blocking inhibitor *N*-ethylmaleimide inhibits the enzyme activity, which points to the importance of some cysteine residues in the molecule (58), maybe for maintaining the functional tertiary structure. To test the importance of the three conserved cysteine residues in *S. cerevisiae* Str3, we sep-

arately mutated these residues and reintroduced the mutated Str3 versions in an Δ*str3* mutant (Fig. 8C). Wild-type Str3 and the C353S and C362S versions restored the ability of *str3* cells to grow on cysteine as the sole sulfur source. In contrast, the Str3(C387S) protein was not functional. Therefore, the Str3 Cys387 residue seems to be essential for cystathione β-lyase function, which supports the importance of redox regulation in the enzyme.

DISCUSSION

A large number of *S. cerevisiae* genes are grouped in families whose products share significant sequence homology. Gene (or protein) families may reflect an evolutionary relationship among their members, although these do not necessarily have overlapping biological functions. Thus, differential compartmentalization may play an important role in functional divergence among family members sharing an enzyme activity, as is the case for the three *S. cerevisiae* monothiol glutaredoxins Grx3/Grx4 (nuclear) and Grx5 (mitochondrial) (49, 59). The three Gto proteins here described are another example of *S. cerevisiae* proteins that share enzymatic properties as GSTs with glutaredoxin activity (25), although they are located at different compartments and probably carry out different biological functions. The fact that to different extents all three *GTO* genes are induced by agents causing oxidative stress probably points to a function in the defense against oxidant conditions for the proteins. However, only the function of Gto1 has been approached in the present study.

The peroxisomal location of Gto1 is relevant, since among the large GST superfamily only two mammalian peroxisomal enzymes had been described to date: a kappa class GST in human cells (50) and a microsomal-type GST in rat liver cells (40). Among the *S. cerevisiae* Gto1 homologues in other fungi, only those from hemiascomycetous yeasts closely related to *S. cerevisiae* (20) are predicted to be peroxisomal as well. This may reflect the functional versatility of peroxisomes along evolution and the fact that only a core of functions is shared between *S. cerevisiae* peroxisomes and those of other eukaryotes (24, 38, 77). The presence of a GST at the peroxisome implies that GSH must be present at the organelle as the substrate of the enzyme. Little is known about partitioning of the GSH pool inside the yeast cell compartments and how the cell regulates subcellular concentrations of GSH (53). However, GSH has been detected in the peroxisomes of methylotrophic yeast *Candida boidinii* (39). Glutathione peroxidases have been described in mammalian peroxisomes (62) and glutathione reductases and glutathione peroxidases in plant peroxisomes (12, 16). Therefore, mechanisms must exist in eukaryotic cells to transport GSH into peroxisomes to act as the substrate for a diversity of antioxidant enzymes.

The present study indicates that Gto1 probably has a specific role in *S. cerevisiae* peroxisomes related to the metabolism of sulfur amino acids. Besides the involvement in the β-oxidation of fatty acids (38), yeast peroxisomes participate in other cellular functions, such as lysine biosynthesis. In fact, Lys1 and Lys4 enzymes have been detected in *S. cerevisiae* peroxisomes (45) and probably Lys12 is also peroxisomal (8), whereas other enzymes of the pathway are cytosolic. Yeast cells negatively affected in peroxisome biogenesis upregulate lysine biosynthesis genes, although peroxisome-deficient *S. cerevisiae* cells are still able to synthesize lysine, probably through alternative reactions (8). The cystathionine β-lyase Str3 is a second example of a peroxisomal *S. cerevisiae* enzyme involved in amino acid metabolism, in this case in the transulfuration pathway from cysteine to homocysteine (Fig. 6). This is the only enzyme in the entire metabolic pathway that has been detected at the peroxisomes (45) and contains a PTS1 sequence at the C terminus (28). Therefore, at least two cell compartments seem to participate in the metabolism and interconversion of sulfur amino acids in *S. cerevisiae*, implying the need for transport of intermediates into and out of peroxisomes. The reason for the organelle compartmentalization of a specific reaction of the pathway might be related to the toxicity of strong reductant intermediates such as cystathionine. Only hemiascomycetous yeasts evolutionary close to *S. cerevisiae* (such as *Kluyveromyces lactis*, *Candida glabrata*, and *Ashbya gossypii*) have a cystathionine β-lyase protein with a C-terminal PTS1 sequence. Therefore, as occurs with lysine biosynthesis, the participation of *S. cerevisiae* peroxisomes in cysteine transulfuration seems to be a recent specialization of some hemiascomycetous yeasts.

The peroxisomal location of the Gto1 GST could parallel that of Str3, and it could have occurred after the whole genome duplication in a hemiascomycete ancestor of *S. cerevisiae* (81) would have allowed functional specialization of the resulting paralogues through differential compartmentalization of the respective products. Gto1 acts as a glutaredoxin that would regulate the redox state of target cysteine residues through its deglutathionylating activity (25). Cys387 in *S. cerevisiae* Str3 could be such a target. In fact, this residue is conserved in fungal cystathionine β-lyase molecules, and here we have shown it to be essential for the transulfuration pathway. Peroxisomes are a source of reactive oxygen species that create an oxidizing environment inside the organelle (16, 62). In these conditions, essential cysteine residues would be oxidized (for instance, through reversible glutathionylation) leading to transient protein inactivation. Gto1 could restore the activity of Str3 (and maybe of other peroxisomal targets) through its redox regulation role. Consistent with this, the absence of Gto1 causes growth defects with cysteine or cystathionine as the only sulfur sources and depleted levels of intracellular GSH. The hypersensitivity of a triple *gtt1 gtt2 gto1* mutant to Cd would result from the combination of reduced GSH levels (this tripeptide is required for Cd detoxification in *S. cerevisiae* by forming Cd-GSH complexes [45, 63]) and the absence of functional Gtt proteins with GSH-conjugating activity. In addition, the imbalance of the metabolic fluxes in the sulfur amino acid metabolic pathway in the Δ*gto1* mutant indirectly causes alterations in the biosynthesis of serine and threonine. In fact, the serine/threonine dehydratase activity of Cha1 besides converting serine into pyruvate (Fig. 6) also converts threonine into 2-oxobutanoate (52). It is therefore to be expected that overexpression of *CHA1* reduces the intracellular pools of both amino acids and makes the cell partially dependent on their external supply. The observed deficiency of the Δ*gto1* mutant for lysine biosynthesis could be a consequence of altered peroxisomal functioning in the mutant (maybe because of the accumulation of reactive oxygen species in the organelle) and the role of this organelle in lysine biosynthesis in *S. cerevisiae* (8).

The present study shows how the defect in a peroxisomal

GST/glutaredoxin function causes a number of phenotypes, probably as a consequence of the alteration in the sulfur amino acid metabolic pathway. The expression of most genes of this pathway is regulated by *S*-adenosylmethionine through the Met4 transcription factor (10, 67). Recently, it has been shown that *S*-adenosylmethionine has to be converted into cysteine for the ubiquitylation-dependent regulation of Met4 activity (48), confirming previous suggestions (33). Met4 participates in two different complexes (together with Met28/Cbf1 or Met31/Met32) for recognition of two respective promoter elements located in *MET* and other genes (5, 43). The genes downregulated in the Δ*gto1* mutant contain one or both elements (Table 2). The list includes *SER3*, *GAP1*, *SAM3*, and *MUP1*, which do not directly mediate the conversion of sulfur into cysteine and methionine (Fig. 6) and contain elements putatively recognized by the Met4/Met31/Met32 complex. However, the downregulation of genes observed in the Δ*gto1* mutant probably is not a consequence of increased cysteine levels, since this would be difficult to reconcile with the observed low levels of GSH in the mutant. Some other metabolite accumulating in the mutant (such as cystathionine) could be the effector of the transcriptional changes. The hypersensitivity of the Δ*gto1* mutant to oxidants could be a consequence of the reduced GSH levels. However, a complex relationship must exist between Gto and Gtt proteins and other hypothetical related functions, for instance with respect to the increased resistance of the mutant lacking all Gto and Gtt proteins to hydroperoxides and 4-nitroquinolone. A partially similar situation has been reported for *S. pombe*, where single or multiple *gst* mutants are hyper-resistant to diamide compared to wild-type cells (72). The induction of other uncharacterized systems protecting against compound toxicity in these conditions could explain these observations. Alternatively, in the case of 4-nitroquinolone the Gto proteins could convert the Gtt2-mediated GSH conjugate to a more toxic derivative. More studies are needed in order to further elucidate the relationship between the peroxisomal regulatory functions mediated by Gto1, sulfur amino acid metabolism, and defense against oxidative stress.

ACKNOWLEDGMENTS

This study was supported by grants from the Spanish Ministry of Education and Science (BFU2004-03167) and the Generalitat de Catalunya (2005SGR00677) to E.H. and from the Swedish Research Council for Science and Technology (2003-3189) to P.S. A.G. was recipient of a postdoctoral grant from the Generalitat de Valencia.

The technical support from Lidia Piedrafita is acknowledged.

REFERENCES

1. **Adamis, P. D. B., D. S. Gomes, M. L. C. C. Pinto, A. D. Panek, and E. C. A. Eleuterio.** 2004. The role of glutathione transferases in cadmium stress. Toxicol. Lett. **154:**81–88.
2. **Armstrong, R. N.** 1997. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem. Res. Toxicol. **10:**2–18.
3. **Avery, S. V.** 2001. Metal toxicity in yeasts and the role of oxidative stress. Adv. Appl. Microbiol. **49:**111–142.
4. **Awasthi, Y. C., G. A. S. Ansari, and S. Awasthi.** 2005. Regulation of 4-hydroxynonenal mediated signaling by glutathione *S*-transferases. Methods Enzymol. **401:**379–407.
5. **Blaiseau, P. L., and D. Thomas.** 1998. Multiple transcriptional activation complexes tether the yeast activator Met4 to DNA. EMBO J. **17:**6327–6336.
6. **Board, P. G., M. Coggan, G. Chelvanagayam, S. Easteal, L. S. Jermiin, G. K. Schulte, D. E. Danley, L. R. Hoth, M. C. Griffor, A. V. Kamath, M. H. Rosner, B. A. Chrunyk, D. E. Perregaux, C. A. Gabel, K. F. Geoghegan, and J. Pandit.** 2000. Identification, characterization, and crystal structure of the omega class glutathione transferases. J. Biol. Chem. **275:**248–24806.
7. **Breitinger, U., T. Clausen, S. Ehlert, R. Huber, B. Laber, F. Schmidt, E. Pohl, and A. Messerchmidt.** 2001. The three-dimensional structure of cystathionine β-lyase from *Arabidopsis* and its substrate specificity. Plant Physiol. **126:**631–642.
8. **Breitling, R., O. Sharif, M. L. Hartman, and S. K. Krisans.** 2002. Loss of compartmentalization causes misregulation of lysine biosynthesis in peroxisome-deficient yeast cells. Eukaryot. Cell **1:**978–986.
9. **Brennan, R. J., and R. H. Schiestl.** 1996. Cadmium is an inducer of oxidative stress in yeast. Mutat. Res. **356:**171–178.
10. **Cherest, H., and Y. Surdin-Kerjan.** 1992. Genetic analysis of a new mutation conferring cysteine auxotrophy in *Saccharomyces cerevisiae*: updating of the sulfur metabolism pathway. Genetics **130:**51–58.
11. **Choi, J. H., W. Lou, and A. Vancura.** 1998. A novel membrane-bound glutathione S-transferase functions in the stationary phase of the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **273:**29915–29922.
12. **Churin, Y., S. Schilling, and H. Börner.** 1999. A gene encoding glutathione peroxidase homologues in *Hordeum vulgare* (barley). FEBS Lett. **459:**33–38.
13. **Claros, M. G., and P. Vincens.** 1996. Computational method to predict mitochondrially imported proteins and their targeting sequences. Eur. J. Biochem. **241:**770–786.
14. **Collinson, E. J., and C. M. Grant.** 2003. Role of yeast glutaredoxins as glutathione *S*-transferases. J. Biol. Chem. **278:**22492–22497.
15. **Collinson, E. J., G. L. Wheeler, E. Ocón-Garrido, A. M. Avery, S. V. Avery, and C. M. Grant.** 2002. The yeast glutaredoxins are active as glutathione peroxidases. J. Biol. Chem. **277:**16712–16717.
16. **Del Rio, L. A., F. J. Corpas, L. M. Sandalio, J. M. Palma, and J. B. Barroso.** 2003. Plant peroxisomes, reactive oxygen metabolism, and nitric oxide. IUBMB Life **55:**71–81.
17. **Deponte, M., and K. Becker.** 2005. Glutathione *S*-transferase from malarial parasites: structural and functional aspects. Methods Enzymol. **401:**241–253.
18. **Diekert, K, A. I. P. M. de Kroon, G. Kispal, G., and R. Lill.** 2001. Isolation and subfractionation of mitochondria from the yeast *Saccharomyces cerevisiae*. Methods Cell Biol. **65:**37–51.
19. **Dormer, U. H., J. Westwater, N. F. McLaren, N. A. Kent, J. Mellor, and D. J. Jamieson.** 2000. Cadmium-inducible expression of the yeast *GSH1* gene requires a functional sulfur-amino acid regulatory network. J. Biol. Chem. **42:**32611–32616.
20. **Dujon, B.** 2005. Hemiascomycetous yeasts at the forefront of comparative genomics. Curr. Opin. Gen. Dev. **15:**1–7.
21. **Erdmann, R., and S. J. Gould.** 2002. Visualization and purification of yeast peroxisomes. Methods Enzymol. **351:**365–381.
22. **Estruch, F.** 2000. Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiol. Rev. **24:**469–486.
23. **Fauchon, M., G. Lagniel, J. C. Aude, L. Lombardia, P. Soularue, C. Petat, G. Marguerie, A. Sentenac, M. Werner, and J. Labarre.** 2002. Sulfur sparing in the yeast proteome in response to sulfur demand. Mol. Cell **9:**713–723.
24. **Gabaldón, T., B. Snel, F. Zimmeren, W. Hemrika, H. Tabak, and M. A. Huynen.** 2006. Origin and evolution of the peroxisomal proteome. Biol. Direct. **1:**8.
25. **Garcerá, A., L. Barreto, L. Piedrafita, J. Tamarit, and E. Herrero.** 2006. *Saccharomyces cerevisiae* cells have three Omega-class glutathione transferases acting as 1-Cys thiol transferases. Biochem. J. **398:**187–196.
26. **Garí, E., L. Piedrafita, M. Aldea, and E. Herrero.** 1997. A set of vectors with the tetracycline-regulatable promoter system for modulated gene expression in *Saccharomyces cerevisiae*. Yeast **13:**837–848.
27. **Gasch, A. P.** 2003. The environmental stress response: a common yeast response to diverse environmental stresses, p. 11–70. *In* S. Hohman and P. W. H. Mager (ed.), Topics in current genetics: yeast stress responses. Springer-Verlag, Berlin, Germany.
28. **Geraghty, M. T., D. Bassett, J. C. Morrell, G. J. Gatto, Jr., J. Bai, B. V. Geisbrecht, P. Hieter, and S. J. Gould.** 1999. Detecting patterns of protein distribution and gene expression in silico. Proc. Natl. Acad. Sci. USA **96:**2937–2942.
29. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:**3065–3073.
30. **Girardini, J., A. Amirante, K. Zemzoumi, and E. Serra.** 2002. Characterization of an omega-class glutathione *S*-transferase from *Schistosoma mansonii* with glutaredoxin-like dehydroascorbate reductase and thiol transferase activities. Eur. J. Biochem. **269:**5512–5521.
31. **Goldstein, A. L., X. Pan, and J. H. McCusker.** 1999. Heterologous *URA3MX* cassettes for gene replacement in *Saccharomyces cerevisiae*. Yeast **15:**507–511.
32. **Goldstein, A. L., and J. H. McCusker.** 1999. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast **15:**1541–1553.
33. **Hansen, J., and P. F. Johannesen.** 2000. Cysteine is essential for transcriptional regulation of the sulfur assimilation genes in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **263:**535–542.
34. **Hayes, J. D., J. U. Flanagan, and I. R. Jowsey.** 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. **45:**51–88.

35. **He, X. J., and J. S. Fassier.** 2005. Identification of novel Yap1p and Skn7p binding sites involved in the oxidative stress response of *Saccharomyces cerevisiae*. Mol. Microbiol. **58:**1454–1467.
36. **Heiland, I., and R. Erdmann.** 2005. Biogenesis of peroxisomes: topogenesis of the peroxisomal membrane and matrix proteins. FEBS J. **272:**2362–2372.
37. **Hesse, H., and R. Hoefgen.** 2003. Molecular aspects of methionine biosynthesis. Trends Plant Sci. **8:**259–262.
38. **Hiltunen, J. K., A. M. Mursula, H. Rottensteiner, R. K. Wierenga, A. J. Kastaniotis, and A. Gurvitz.** 2003. The biochemistry of peroxisomal β-oxidation in the yeast *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. **27:**35–64.
39. **Horiguchi, H., H. Yurimoto, N. Kato, and Y. Sakai.** 2001. Antioxidant system within yeast peroxisome: biochemical and physiological characterization of CbPmp20 in the methylotrophic yeast *Candida boidinii*. J. Biol. Chem. **276:** 14279–14288.
40. **Islinger, M., G. H. Lüers, H. Zischka, M. Ueffing, and A. Völkl.** 2006. Insights into the membrane proteome of rat liver peroxisomes: microsomal glutathione-*S*-transferase is shared by both subcellular compartments. Proteomics **6:**804–816.
41. **Jakubowski, W., T. Bilinski, and G. Bartosz.** 2000. Oxidative stress during aging of stationary cultures of the yeast *Saccharomyces cerevisiae*. Free Radic. Biol. Med. **28:**659–664.
42. **Kumar, A., L. John, M. M. Alam, A. Gupta, G. Sharma, B. Pillai, and S. Sengupta.** 2006. Homocysteine- and cysteine-mediated growth defect is not associated with induction of oxidative stress response genes in yeast. Biochem. J. **396:**61–69.
43. **Kuras, L., R. Barbey, and D. Thomas.** 1997. Assembly of a bZIP-bHLH transcription activation complex: formation of the yeast Cbf1-Met4-Met28 complex is regulated through Met28 stimulation of Cbf1 DNA binding. EMBO J. **16:**2441–2451.
44. **Lametschwandtner, G., C. Brocard, M. Fransen, P. van Veldhoven, J. Berger, and A. Hartig.** 1998. The difference in recognition of terminal tripeptides as peroxisomal targeting signal 1 between yeast and human is due to different affinities of their receptor Pex5p to the cognate signal and to residues adjacent to it. J. Biol. Chem. **273:**33635–33643.
45. **Li, Z., Y. Lu, R. Zhen, M. Szczypka, D. Thiele, and P. A. Rea.** 1997. A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis(glutathionate)cadmium. Proc. Natl. Acad. Sci. USA **94:**42–47.
46. **Mannervik, B., P. G. Board, J. D. Hayes, I. Listowsky, and W. R. Pearson.** 2005. Nomenclature for mammalian soluble glutathione transferases. Methods Enzymol. **401:**1–8.
47. **McGoldrick, S., S. M. O'Sullivan, and D. Sheehan.** 2005. Glutathione transferase-like proteins encoded in genomes of yeasts and fungi: insights into evolution of a multifunctional protein superfamily. FEMS Microbiol. Lett. **242:**1–12.
48. **Menant, A., P. Baudouin-Cornu, C. Peyraud, M. Tyers, and D. Thomas.** 2006. Determinants of the ubiquitin-mediated degradation of the Met4 transcription factor. J. Biol. Chem. **281:**11744–11754.
49. **Molina, M. M., G. Bellí, M. A. de la Torre, M. T. Rodríguez-Manzaneque, and E. Herrero, E.** 2004. Nuclear monothiol glutaredoxins of *Saccharomyces cerevisiae* can function as mitochondrial glutaredoxins. J. Biol. Chem. **279:** 1923–51930.
50. **Morel, F., C. Rauch, E. Petit, A. Piton, N. Theret, B. Coles, and A. Guillouzo.** 2004. Gene and protein characterization of the human glutathione *S*-transferase kappa and evidence for a peroxisomal localization. J. Biol. Chem. **279:**16246–16253.
51. **Oakley, A. J.** 2005. Glutathione transferases: new functions. Curr. Opin. Struct. Biol. **15:**716–723.
52. **Petersen, J. G., M. C. Kielland-Brandt, T. Nilsson-Tillgren, C. Bornaes, and S. Holmberg.** 1988. Molecular genetics of serine and threonine catabolism in *Saccharomyces cerevisiae*. Genetics **119:**527–534.
53. **Pócsi, I., R. A. Prade, and M. J. Penninckx.** 2004. Glutathione, altruistic metabolite in fungi. Adv. Microb. Physiol. **49:**1–76.
54. **Puig, O., F. Caspary, G. Rigaut, B. Rutz, E. Bouveret, E. Bragado-Nilsson, M. Wilm, and B. Seraphin.** 2001. The tandem affinity purification (TAP) method: a general procedure of protein complex purification. Methods **24:** 218–229.
55. **Purdue, P. E., and P. B. Lazarow.** 2001. Peroxisome biogenesis. Annu. Rev. Cell Dev. Biol. **17:**701–752.
56. **Rai, R., and T. G. Cooper.** 2005. In vivo specificity of Ure2 protection from heavy metal ion and oxidative cellular damage in *Saccharomyces cerevisiae*. Yeast **22:**343–358.
57. **Rai, R., J. J. Tate, and T. G. Cooper.** 2003. Ure2, a prion precursor with homology to glutathione *S*-transferase, protects *Saccharomyces cerevisiae* cells from heavy metal ion and oxidant toxicity. J. Biol. Chem. **278:**12826–12833.
58. **Ravanel, S., D. Job, and R. Douce.** 1996. Purification and properties of cystathionine β-lyase from *Arabidopsis thaliana* overexpressed in *Escherichia coli*. Biochem. J. **320:**383–392.
59. **Rodríguez-Manzaneque, M. T., J. Tamarit, J. G. Bellí, J. Ros, and E. Herrero.** 2002. Grx5 is a mitochondrial glutaredoxin required for the maturation of iron/sulfur enzymes. Mol. Biol. Cell **13:**1109–1121.
60. **Rouimi, P., P. Anglade, A. Benzekri, P. Costet, L. Debrauwer, T. Pineau, and J. Tulliez.** 2001. Purification and characterization of a glutathione *S*-transferase Omega in pig: evidence for two distinct organ-specific transcripts. Biochem. J. **358:**257–262.
61. **Schafer, H., K. Nau, A. Sickmann, R. Erdmann, and H. E. Meyer.** 2001. Identification of peroxisomal membrane proteins of *Saccharomyces cerevisiae* by mass spectrometry. Electrophoresis **22:**2955–2968.
62. **Schrader, M., and H. D. Fahimi.** 2004. Mammalian peroxisomes and reactive oxygen species. Histochem. Cell Biol. **122:**383–393.
63. **Sharma, K. G., D. L. Mason, G. Liu, P. A. Rea, A. K. Bachhawat, and S. Michaelis.** 2002. Localization, regulation, and substrate transport properties of Bpt1p, a *Saccharomyces cerevisiae* MRP-type ABC transporter. Eukaryot. Cell **1:**391–400.
64. **Sheehan, D., G. Meade, V. M. Foley, and C. A. Dowd.** 2001. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J. **360:**1–16.
65. **Sherman, F.** 2002. Getting started with yeast. Methods Enzymol. **350:**3–41.
66. **Tatchell, K., and L. C. Robinson.** 2002. Use of green fluorescent protein in living yeast cells. Methods Enzymol. **351:**661–683.
67. **Thomas, D., and Y. Surdin-Kerjan.** 1997. Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **61:**503–532.
68. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
69. **Toledano, M. B., A. Delaunay, B. Biteau, D. Spector, and D. Azevedo.** 2003. Oxidative stress responses in yeast, p. 241–303. *In* S. Hohman and P. W. H. Mager (ed.), Topics in current genetics: yeast stress responses. Springer-Verlag, Berlin, Germany.
70. **Townsend, D. M., V. L. Findlay, and K. D. Tew.** 2005. Glutathione S-transferases as regulators of kinase pathways and anticancer drug targets. Methods Enzymol. **401:**287–307.
71. **Van Helden, J.** 2003. Regulatory sequence analysis tools. Nucleic Acids Res. **31:**3593–3596.
72. **Veal, E. A., W. M. Toone, N. Jones, and B. A. Morgan.** 2002. Distinct roles for glutathione S-transferases in the oxidative stress response in *Schizosaccharomyces pombe*. J. Biol. Chem. **277:**35523–35531.
73. **Veenhuis, M., F. A. Salomons, and I. J. Van der Klei.** 2000. Peroxisome biogenesis and degradation in yeast: a structure/function analysis. Microsc. Res. Technol. **51:**584–600.
74. **Viladevall, L., R. Serrano, A. Ruiz, G. Domenech, J. Giraldo, A. Barceló, and J. Ariño.** 2004. Characterization of the calcium-mediated response to alkaline stress in *Saccharomyces cerevisiae*. J. Biol. Chem. **279:**43614–43624.
75. **Vuilleumier, S.** 1997. Bacterial glutathione S-transferases: what are they good for? J. Bacteriol. **179:**1431–1441.
76. **Wach, A., A. Brachat, R. Pöhlmann, and P. Philippsen.** 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **13:**1793–1808.
77. **Wanders, R. J. A., and H. R. Waterman.** 2006. Biochemistry of mammalian peroxisomes revisited. Annu. Rev. Biochem. **75:**295–332.
78. **Warringer, J., and A. Blomberg.** 2003. Automated screening in environmental arrays allows analysis of quantitative phenotypic profiles in *Saccharomyces cerevisiae*. Yeast **20:**53–67.
79. **Weiner, M. P., and L. Costa.** 1995. Rapid PCR site-directed mutagenesis, p. 613–621. *In* C. W. Dieffenbach and G. S. Dveksler (ed.), PCR primer: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
80. **Whitbread, A. K., A. Masoumi, N. Tetlow, E. Schmuck, M. Coggan, and P. G. Board.** 2005. Characterization of the Omega-class of glutathione transferases. Methods Enzymol. **401:**78–99.
81. **Wong, S., G. Butler, and K. H. Wolfe.** 2002. Gene order evolution and paleopolyploidy in hemiascomycete yeasts. Proc. Natl. Acad. Sci. USA **387:** 708–713.
82. **Yi, E. C., M. Marelli, H. Lee, S. O. Purvine, R. Aebersold, J. D. Aitchison, and D. R. Goodlett.** 2002. Approaching complete peroxisome characterization by gas-phase fractionation. Electrophoresis **23:**3205–3216.