Glutathione as an endogenous sulphur source in the yeast *Saccharomyces cerevisiae*

MARC T. ELSKENS, CHARLES J. JASPERS and MICHEL J. PENNINCKX*

Laboratoire de Microbiologie de l'Université libre de Bruxelles, Unité de Physiologie et Ecologie Microbienne, Institut de Recherches du CERIA, 1 Av. E. Gryson, B-1070, Bruxelles, Belgium

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Glutathione-deficient mutants (*gshA*) of the yeast *Saccharomyces cerevisiae*, impaired in the first step of glutathione (GSH) biosynthesis were studied with respect to the regulation of enzymes involved in GSH catabolism and cysteine biosynthesis. Striking differences were observed in the content of the sulphur amino acids when *gshA* mutants were compared to wild-type strains growing on the same minimal medium. Furthermore, all mutants examined showed a derepression of γ -glutamyltranspeptidase (γ -GT), the enzyme initiating GSH degradation. However, γ -cystathionase and cysteine synthase were unaffected by the GSH deficiency as long as the nutrient sulphate source was not exhausted. The results suggest that the mutants are probably not impaired in the sulphate assimilation pathway, but that the γ -glutamyl cycle could play a leading role in the regulation of the sulphur fluxes. Studies of enzyme regulation showed that the derepression of γ -GT observed in the *gshA* strains was most probably due to an alteration of the thiol status. The effectors governing the biosynthesis of cysteine synthase and γ -cystathionase seemed different from those playing a role in γ -GT regulation and it was only under conditions of total sulphate deprivation that all these enzymes were derepressed. As a consequence the endogenous pool of GSH was used in the synthesis of cysteine. GSH might, therefore, fulfil the role of a storage compound.

Introduction

The thiol tripeptide glutathione (GSH) accounts for about 1% of the dry weight of the yeast *Saccharomyces cerevisiae* and plays a leading part in the detoxification of endogenous oxoaldehydes (Penninckx *et al.*, 1983) and the regulation mechanisms of drug bioreduction (Elskens *et al.*, 1988) in this organism. A role for GSH in sulphur metabolism was apparently not previously envisaged in studies of yeast metabolism (Jones & Fink, 1982; Ono *et al.*, 1988). GSH was, however, cited as a growth factor for micro-organisms (see Penninckx & Jaspers, 1982). It also appears to be an alternative source of sulphur for morphogenesis of *Bacillus cereus* spores (Cheng *et al.*, 1973) and vegetative cells of a species of cyanobacterium (Giddings *et al.*, 1981). Observations by several investigators (see Rennenberg, 1982) are consistent with the idea that GSH functions as a storage form of reduced sulphur in plants; this function has also been proposed

for animal cells (Kosower & Kosower, 1978; Reed *et al.*, 1983).

Several lines of evidence indicate that GSH metabolism proceeds in many eukaryotic cells via the γ -glutamyl cycle (Meister, 1983). In *S. cerevisiae*, however, GSH catabolism appears to be mediated by γ -glutamyltranspeptidase (γ -GT) and cysteinylglycine dipeptidase only, and leads to the formation of glutamate and γ -glutamyl amino acids (Jaspers *et al.*, 1985). The physiological role of yeast transpeptidation reactions is not well understood. γ -GT and GSH were initially thought to be involved in the bulk absorption of amino acids by *S. cerevisiae*, but these claims have been contradicted (Robins & Davies, 1981; Jaspers & Penninckx, 1981; Payne & Payne, 1984). In the course of an investigation into the biochemical characterization of GSH-deficient yeast mutants, we observed that these mutants showed a derepression of γ -GT. Efforts were made to understand the physiological significance of this regulation pattern by using mutants impaired in the first step of GSH biosynthesis. It was of interest to know if the regulation of other sulphur-related enzymes was also altered in these strains. In this paper, we present evidence that GSH might serve as an endogenous sulphur reservoir when *S.*

Abbreviations: BSO, buthionine-(S,R)-sulphoximine; mBRB, monobromobimane; GSH, glutathione; GSSG, oxidized glutathione; γ -GT, γ -glutamyltranspeptidase.

cerevisiae cells are deprived of any external sulphur nutrient. If the γ -glutamyl cycle does provide the sulphur for amino acid biosynthesis, it is probably regulated. The regulatory controls of γ -GT and the enzymes of the transsulphuration pathway are discussed in the light of current concepts of sulphur metabolism in *S. cerevisiae*.

Methods

Strains. Isogenic wild-type strains Σ 1278b (*MAT α*) and 3962c (*MAT α*) used in growth and genetic experiments were from this laboratory (Grenson *et al.*, 1966). GSH-deficient (*Gsh⁻*) yeast strains (Kistler *et al.*, 1986) derived from the wild-type strain BK RB1 were kindly provided by Dr Eckardt and colleagues. In order to eliminate auxotrophic mutations, the original isolate RB1/8 (*MAT α Trp⁻ Ilv⁻ Ade⁻ Gsh⁻*) was crossed with strain 3962c. Sporulated diploids obtained after growth on acetate as a carbon source were dissected according to the classical procedure. 5/2 tetrads showing a prototrophic character were obtained after selection on a minimal medium containing (NH₄)₂SO₄ and glucose as the sole sources of nitrogen and carbon respectively. A prototrophic spore progeny showing a clear 2:2 segregation for GSH deficiency was obtained by successive back-crossing of an otherwise prototrophic *Gsh⁻* segregant with strain Σ 1278b. The enzymic defect was identified at the level of γ -glutamylcysteine synthetase (*gshA*). Three segregants – *gshA-1*, *gshA-2* and *gshA-3* – were chosen for the studies described below.

Growth conditions. Orbital shake batch cultures (0.5 and 1 litre) were inoculated from aerobic, stationary phase, glucose-grown precultures and incubated overnight at 29 °C to obtain *S. cerevisiae* cells in the exponential growth phase. The cells were harvested at a maximum of 0.5–0.6 mg dry wt ml⁻¹ to obtain convenient samples for the determination of enzyme specific activities and metabolic intermediates. A minimal medium (MM) containing 50 mM-(NH₄)₂SO₄ and 3% (w/v) glucose as sole sources of nitrogen and carbon respectively was used as the standard medium (Messenguy, 1976). A solution containing MgCl₂ (3 mM), KH₂PO₄ (7.5 mM), CaCl₂ (3 mM), NaCl (9 mM), citric acid (50 mM) and KOH (160 mM) was used as a sulphur-deficient mineral medium for the experiments reported in Fig. 3. Numerous precautions were taken to avoid contamination with sulphur during the preparation of the medium. All glassware was acid cleaned and rinsed several times with double-deionized water (Milli-Q) before use. The chemicals were of reagent grade quality. The medium was prepared daily and dispensed into serum bottles to limit sulphide contamination from the ambient environment. The bottles were closed by butyl rubber stoppers, sealed with aluminium caps and then autoclaved. In the shifting experiments shown in Fig. 3, the cells were transferred under sterile conditions to the sulphur-deficient medium after rapid Millipore filtration (0.45 μ m HAWP).

Enzyme assays. Cells were harvested at 4 °C by centrifugation at 6000 *g* for 10 min, washed twice with an appropriate buffer for the enzyme determination and resuspended in the same buffer. The cells were ruptured by passage through a French pressure cell. The homogenate was centrifuged (27000 *g* for 15 min) and the supernatant fluid was used as the enzyme source.

GSH synthetase (EC 6.3.2.3), γ -glutamyltranspeptidase (EC 2.3.2.2) and cysteinylglycine dipeptidase activities were assayed according to Jaspers *et al.* (1985). γ -Glutamylcysteine synthetase was assayed following Kistler *et al.* (1990). γ -Cystathionase (EC 4.4.1.1) was assayed by the following procedure (F. Ramos, personal communication): 0.5 ml of enzyme solution (up to 1 mg protein),

previously filtered on Sephadex G-25, was incubated at 30 °C for up to 5 min with 20 mM-cystathionine (0.1 ml) in the presence of potassium phosphate buffer pH 8.0 (100 mM, 0.4 ml) containing 50 μ M-pyridoxal phosphate. The reaction was stopped by addition of 50 μ l 50% (w/v) trichloroacetic acid. After centrifugation for 5 min at 7000 *g*, L-cysteine was estimated in 0.5 ml supernatant by the procedure of Gaitonde (1967). Units of γ -cystathionase activity (U) are μ moles of cysteine produced per hour. *O*-Acetyl-L-serine, the substrate for the assays of *O*-serine transsulphydrase (cysteine synthase; EC 4.2.99.8) was prepared according to Sheehan *et al.* (1956). Cysteine synthase was estimated by the following procedure: 0.3 ml of enzyme solution (up to 1 mg protein), previously filtered in Sephadex G-25, was incubated at 30 °C for up to 15 min with 10 mM-Na₂S (0.25 ml) and 100 mM-*O*-acetyl-L-serine (0.1 ml) in the presence of Tris/HCl buffer pH 7.4 (200 mM, 0.35 ml) containing 1 mM-Na₂EDTA. The reaction was stopped by addition of 50 μ l 50% (w/v) trichloroacetic acid. After centrifugation for 5 min at 7000 *g*, L-cysteine was estimated in 0.5 ml supernatant by the procedure of Gaitonde (1967). Units (U) are μ moles of cysteine produced per hour.

All enzyme assay conditions (pH, temperature, substrate concentration, incubation time) were optimized in preliminary experiments not reported here.

Protein was estimated by the Lowry method.

Determination of metabolic intermediates. The method of Fahey *et al.* (1987) was adapted for the estimation of low-molecular-mass thiols in *S. cerevisiae*. Cells ($\pm$ 100 mg dry wt) were harvested at 4 °C by centrifugation at 6000 *g* for 5 min, washed twice with HEPPS buffer (10 mM, pH 8.0), and flushed for 10 min with argon in septum-capped vial (serum tubes). Argon-saturated aqueous 100 mM-HEPPS/NaOH (0.5 ml, pH 8.0) containing 5 mM-EDTA and 2 mM-monobromobimane (mBRB) was then added and the suspension, protected from light with aluminium foil, was allowed to stand for 5 min at 20 °C. Argon-saturated acetonitrile (0.5 ml) was added and the sample was incubated for 15 min at 20 °C. The vial was opened and the cells were ruptured by sonication for 10 min with a Vibra Cell VC500 ultrasonic processor (Sonics Materials). Glacial acetic acid (1%, v/v, final concentration) was added to the homogenate cooled in ice and the suspension was centrifuged at 27000 *g* for 15 min. The supernatant was clarified on a Centrifo membrane (Amicon) for 10 min at 1500 *g* prior to HPLC analysis. For control samples, 2 mM-5,5'-dithiobis(2-nitrobenzoic acid) was substituted for mBRB during homogenization. Just after sonication, mBRB was added to a final concentration of 2 mM and the mixture was allowed to react for 5 min at room temperature. Further processing of control samples was as described above. Analyses were done by HPLC (Waters) using a C18 Bondapak column according to Fahey & Newton (1986).

A standard procedure (Ramos *et al.*, 1970) was used for the extraction of GSH and total amino acid pools in *S. cerevisiae*. Aliquots were passed through a Beckman 120 c amino acid analyser. Reduced and oxidized glutathione (GSSG) were also estimated according to Eyer & Podhradsky (1986) as indicated in the text.

Results

Effect of GSH deficiency on metabolic intermediates of sulphur metabolism

GSH was the major non-protein thiol in *S. cerevisiae*, even in the mutant strains where the total GSH content was only 7% of the wild-type value (Table 1). The other thiols were cysteine, γ -glutamylcysteine and CoA-SH. In

Table 1. Effects of GSH deficiency on metabolic intermediates and enzymes involved in the general sulphur metabolism of *S. cerevisiae*

Cells of *S. cerevisiae* were harvested during the exponential growth phase. The values reported (means $\pm$ SD; $n = 3$) were determined as described in Methods.

	Wild-type Σ 1278b	Mutant		
		<i>gshA-1</i>	<i>gshA-2</i>	<i>gshA-3</i>
Metabolic intermediate [nmol (mg dry wt) ⁻¹]*				
Cysteine	0.04 ± 0.01	0.19 ± 0.09	0.13 ± 0.05	0.12 ± 0.05
γ-Glutamylcysteine	0.09 ± 0.03	<0.01	<0.01	<0.01
CoA-SH	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.03	0.07 ± 0.02
U ₄ †	<0.01	0.13 ± 0.04	0.12 ± 0.03	0.10 ± 0.04
GSH	7 ± 1	0.5 ± 0.10	0.5 ± 0.07	0.5 ± 0.13
GSH + GSSG‡	9 ± 0.5	0.6 ± 0.04	0.7 ± 0.04	0.6 ± 0.03
GSSG‡	0.2 ± 0.02	<0.02	<0.02	<0.02
Methionine + cystathionine§	0.8 ± 0.07	2 ± 0.7	2 ± 0.9	3 ± 1
Serine	7 ± 0.8	10 ± 0.9	11 ± 1	11 ± 2
Threonine	8 ± 1	13 ± 2	12 ± 1	14 ± 2
Enzyme activity [nmol h ⁻¹ (mg protein) ⁻¹]				
γ-Glutamylcysteine synthetase	66 ± 7	7 ± 1	5 ± 1	6 ± 1
Glutathione synthetase	216 ± 7	189 ± 10	201 ± 5	214 ± 9
γ-GT	44 ± 4	90 ± 6	109 ± 7	97 ± 9
Dipeptidase	80 ± 10	78 ± 9	93 ± 7	81 ± 5
Cysteine synthase	260 ± 16	300 ± 12	240 ± 18	350 ± 24
γ-Cystathionase	350 ± 32	420 ± 27	330 ± 27	370 ± 39
Generation time (min)	120	189	203	190

* Values for amino acids not affected by the mutation are not shown; a significant difference was, however, noted for ornithine and arginine.

† U₄ denotes an unidentified thiol derivative having a retention time of 4 min in the tetrabutylammonium ion pairing system (Fahey & Newton, 1986). The concentration was estimated using the fluorescence yield factor for the glutathione biman derivative.

‡ Reduced and oxidized glutathione were also measured according to Eyer & Podhradsky (1986) as described in Methods.

§ The assay technique used did not discriminate between methionine and cystathionine.

the GSH-deficient strains, however, the concentration of cysteine was about three times higher than in the wild-type and an unidentified thiol (U₄) was also detected. In contrast, γ -glutamylcysteine was not detected in the mutants. This is most probably due to the greatly lowered activity of γ -glutamylcysteine synthetase in the *Gsh*⁻ phenotype (Table 1). Some underestimation of the total thiol concentration measured by HPLC was likely since glutathione recovery was generally lower ($\pm$ 20%) than with the standard procedure (see Table 1 and Methods). This could be due to differences in extraction yields between the two protocols. Oxidized glutathione (GSSG) represented less than 2% of the GSH content in the wild-type strain and was not detected in the mutants. During the exponential growth phase the thiol:disulphide balance (GSH:GSSG) remained almost constant for strain Σ 1278b, ranging between 50 and 60. These values are similar to those obtained for other eukaryotic cells (Kosower & Kosower, 1978). As previously shown, GSH deficiency leads to pleiotropic phenotypes of the mutants (Kistler *et al.*, 1986). The lowered GSH levels in the haploid *Gsh*⁻ mutants were related to an extension of the

lag phase and a decrease in the growth rate when cells were cultivated on MM medium (Table 1).

Besides thiol compounds, the intracellular concentrations of certain amino acids were also affected by the mutation leading to GSH deficiency, especially when *S. cerevisiae* cells were grown on MM medium using ammonia as the nitrogen source (Table 1). Among the amino acids affected were threonine, serine, methionine or (and) cystathionine. Since all these are involved in the sulphur metabolism of *S. cerevisiae*, and since GSH represents more than 95% of the low-molecular-mass thiol pool in yeast, a link between GSH and general sulphur metabolism was considered. The sulphur atom of GSH could thus be transferred from cysteine to methionine via the γ -glutamyl cycle and the cystathionine pathway. The existence of a complete bidirectional transsulphuration pathway in yeast has been shown previously (Massetot & de Robichon-Szulmajster 1975; Masselot & Surdin-Kerjean, 1977).

Further investigations have emphasized a requirement for studies of enzyme regulation and GSH metabolism in order to support this assessment.

Fig. 1. Effect of GSH on the differential rate of γ -GT synthesis in a GSH-deficient strain of *S. cerevisiae*. Experimental procedures were as described in Methods. $\blacktriangle$, *gshA-1*; $\triangle$, *gshA-1* (+ 3 mM-GSH); $\bullet$, Σ 1278b; $\circ$, Σ 1278b (+ 3 mM-GSH).

Fig. 2. (a) Effect of the addition of 1 mM-BSO (at zero time) on the intracellular pool of GSH (○) and the specific activity of γ -GT (●) in the wild-type strain Σ 1278b. Experimental procedures were as described in Methods. (b) Relationship between γ -GT activity and cellular GSH content; inset, double reciprocal plot.

Regulation of enzyme synthesis in relation to GSH deficiency

As shown in Table 1, GSH deficiency has a noticeable influence on the specific activity of γ -GT. Indeed, in the Gsh⁻ phenotype, the specific activity of γ -GT was about double that in the wild-type strain growing on the same medium. There was no apparent effect on cysteinylglycine dipeptidase, the enzyme immediately downstream that catalyses the release of cysteine. In fact, it was previously observed (Jaspers *et al.*, 1985) that this activity is shared by several peptidases constitutively produced by *S. cerevisiae*. The specific activities of the enzymes involved in cysteine biosynthesis, γ -cystathionase and cysteine synthase, were unaffected by the mutation leading to GSH deficiency (Table 1).

The effect of GSH on the differential rate of γ -GT synthesis was investigated. This thiol is apparently taken up unchanged by cells of *S. cerevisiae* (Jaspers *et al.*, 1985) and does not affect the growth rate at concentrations up to at least 3 mM. As shown in Fig. 1, addition of GSH to a culture of a deficient strain reduced the differential rate of γ -GT synthesis to a value close to that of the wild-type. The values [nmol h⁻¹ (mg protein)⁻¹] for the specific activities were as follows: *gshA-1*, 99 ± 5 ; *gshA-1* (+ GSH), 60 ± 2 ; Σ 1278b, 47 ± 2 ; Σ 1278b (+ GSH), 44 ± 2 .

Buthionine-(S,R)-sulphoximine (BSO), a specific transition-state inhibitor of γ -glutamylcysteine synthetase (Griffith & Meister, 1979), lowered the GSH level in cells of *S. cerevisiae* (Fig. 2). Depletion with BSO enabled us to check the effect of GSH on γ -GT biosynthesis in the wild-type strain Σ 1278b. The growth rate of the strain was apparently unaffected by the analogue at a concentration of 1 mM, but the specific activity of γ -GT increased progressively while the intracellular GSH pool declined (Fig. 2a). The rate of GSH disappearance was used as a measure of GSH utilization. The log-linear regression applied to the data yielded a half-life time ($t_{1/2}$) of 105 ± 4 min ($r = 0.990$, $n = 16$), which was close to the rate of dilution due to growth (see Fig. 2a and Table 1). This is not surprising, since the turnover rate of glutathione during growth on MM medium is exceedingly slow ($t_{1/2}$ about 900 min; see Jaspers *et al.*, 1985). Fig. 2(b) shows the relationship between the level of γ -GT activity and the internal GSH content. Since a linear dependence is observed in a double reciprocal plot, it is possible that regulation of γ -GT could display an element of saturation kinetics, but this problem requires further investigation. For concentrations of GSH higher than 4 nmol (mg dry wt)⁻¹ there were no apparent effects on the level of the enzyme. This could explain why the differential rate of γ -GT synthesis was unaffected in a wild-type strain growing on medium supplemented with GSH (Fig. 1). It seems, therefore, that the enzyme derepression observed in *gshA* mutants is unlikely to result from a mutation in regulatory genes, but rather is due to an alteration of the thiol status.

GSH pool changes

The role of GSH as an endogenous sulphur source for *S. cerevisiae* is shown in Fig. 3. Cells preincubated in MM medium containing sulphate as a sulphur source were transferred into a fresh medium devoid of any sulphur nutrient. Under the experimental conditions of Fig. 3(a), the wild-type strain Σ 1278b continued to grow for 2 h before reaching stationary phase (not shown). During this time the GSH pool decreased to about 10% of its normal value and methionine + cystathionine remained

Fig. 3. Effect of sulphate deprivation on (a) pools of sulphur amino acids [nmol (mg dry wt)⁻¹] and (b) specific activities of enzymes [nmol h⁻¹ (mg protein)⁻¹] in the wild-type strain Σ 1278b. At zero time, the cells were transferred into a sulphate-deficient medium as described in Methods. $\circ$, GSH; $\bullet$, methionine+cystathionine; $\square$, γ -GT; $\blacktriangle$, γ -cystathionase; $\triangle$, cysteine synthase.

Fig. 4. Effect of exogenous L-methionine (1 mg ml⁻¹) on the internal pool of GSH in the wild-type strain Σ 1278b. Experimental procedures were as described in Methods; the time of methionine addition is indicated by an arrow. $\circ$, Growth curve (optical density at 660 nm); $\bullet$, GSH pool; $\triangle$, γ -GT activity.

unaffected (Fig. 3a). After the GSH depletion phase, a net decrease in the concentration of the sulphur compounds was observed. Moreover, γ -GT, cysteine synthase and γ -cystathionase were derepressed when the intracellular concentration of GSH fell below 4 nmol (mg dry wt)⁻¹ (Fig. 4b). The rate of glutathione disappearance was calculated for the first 2 h after the shifting experiment shown in Fig. 3: the $t_{1/2}$ value was 38 ± 1 min ($r = 0.998$, $n = 4$).

Additional evidence that GSH functions as a storage form of reduced sulphur was obtained from studies with L-methionine. Incubation of wild-type cells with L-methionine induced a transitory stimulation of GSH biosynthesis (Fig. 4). A very slight effect was observed for GSH in the mutant strains (not illustrated). Previous studies have shown that methionine is taken up by *S. cerevisiae* cells but cannot serve as a nitrogen source (Gits & Grenson, 1967). Methionine in excess is rapidly

converted into S-adenosyl-L-methionine which is accumulated in yeast vacuoles (Schlenk & DePalma, 1957; Nakamura & Schlenk, 1974). The experiment reported above suggests that the sulphur atoms of methionine could be incorporated into GSH via the cystathionine pathway and the γ -glutamyl cycle. The intracellular concentration of GSH reflects the equilibrium between synthesis and degradation, the latter catalysed mainly by γ -GT. Since the level of γ -GT activity was unaffected by methionine under the experimental conditions illustrated in Fig. 4, the transitory nature of GSH accumulation could be explained by a slow response of one or several enzyme(s) to regulatory signals, because the γ -glutamylcysteine synthetase reaction is the rate limiting step in GSH biosynthesis and is feedback inhibited by GSH under conditions similar to those that prevail *in vivo* (see in Meister & Anderson, 1983).

Discussion

A working model for the main fluxes of sulphur in *S. cerevisiae* is shown in Fig. 5. This model links data published on sulphur metabolism in *S. cerevisiae* (Jones & Fink, 1982; Ono *et al.*, 1988) with our results on GSH metabolism. Potentially, two pathways exist for the synthesis of cysteine. The first (reactions 1 and 2) proceeds by acetylation of serine to yield O-acetylserine, followed by a sulphydrylation. The latter step can be catalysed by at least two cysteine synthase isoenzymes (Yamagata, 1980). The second pathway proceeds from homocysteine and involves the cystathionine biosynthesis cycle (reactions 5 to 8; Masselot & de Robichon-Szulmajster, 1975; Masselot & Surdin-Kerjean, 1977). The final release of cysteine is catalysed by γ -cystathionase. It is also assumed that the γ -glutamyl cycle (reactions 14 to 17) can function to meet the intracellular requirement for cysteine, and is thus involved in the overall regulation of sulphur metabolism. The suppression of this regulating effect in the Gsh⁻ haploids could provide a plausible explanation for the high level of the transsulphuration intermediates (Table 1). In the presence of sulphate as a nutrient, most of the excess sulphur is incorporated into glutathione. Under these conditions, γ -GT activity is low and the turnover rate of GSH is exceedingly slow (Fig. 2 and Jaspers *et al.*, 1985). When cells are deprived of sulphate, GSH is apparently able to serve as an internal sulphur source until it reaches a residual concentration of about 10% of its normal value. An increase in the specific activity of γ -GT is attained by derepressing synthesis of the enzyme and, as a consequence, the turnover rate of GSH increases (Fig. 3). The model requires that derepression of the bidirectional transsulphuration pathway (reactions 5 to 8 in Fig. 5)

Fig. 5. A model for the main fluxes of sulphur in *S. cerevisiae*. (1) Serine acetyltransferase; (2) cysteine synthase; (3) homoserine acetyltransferase; (4) homocysteine synthase; (5) γ -cystathionine synthase; (6) γ -cystathionase; (7) β -cystathionase; (8) β -cystathionine synthase; (9) homocysteine methyltransferase; (10) *S*-adenosylmethionine synthetase; (11) *S*-adenosylmethionine demethylase; (12) adenosylhomocysteinase; (13) sulphate-reducing pathway; (14) γ -glutamylcysteine synthetase; (15) GSH synthetase; (16) γ -GT; (17) cysteinylglycine dipeptidase.

leads to a more active transfer of the sulphur atom from cysteine to methionine and vice versa. Pasewski *et al.* (1984) suggested that GSH, in addition to cysteine, could act as an effector regulating the biosynthesis of the enzymes of sulphur metabolism in the fungus *Aspergillus nidulans*. Here we have found that in *S. cerevisiae*, the effectors governing the biosynthesis of the transsulphuration enzymes are apparently different from those playing a role in γ -GT regulation, since γ -cystathionase and cysteine synthase remain unaffected by the GSH deficiency in *gshA* mutants. Derepression of γ -GT correlates best with a decrease in the GSH pool among the sulphur compounds examined (Table 1 and Fig. 2). This is not the case for γ -cystathionase and cysteine synthase. The two regulatory circuits appear to be distinct and it is only in conditions of total sulphate deprivation that all these enzymes are derepressed.

Interestingly, only 90% of the GSH pool is mobilizable to serve as an endogenous sulphur source. From experiments with BSO, approximately 10% of the wild-

type GSH content is the minimum critical level (Fig. 2). Cells containing about 1 nmol GSH (mg dry wt)⁻¹ or more are apparently able to grow normally; we do not know if a complete lack of GSH is lethal in *S. cerevisiae* since the growth rate decreases dramatically below this value. Kistler *et al.* (1986) reported that a residual GSH level of 1% of the wild-type value, as found in GSH-deficient mutants (*gshA*), leads to serious impairments of the plating efficiencies of the cells and their respiration ability accompanied by a loss of mitochondrial DNA. Some eukaryotic cells have a GSH mitochondrial pool which is stable and physiologically distinct from the cytoplasmic pool (Meredith & Reed, 1982). During severe drug intoxication, extensive depletion of the cytoplasmic GSH pool had less damaging consequences than depletion of the mitochondrial pool (Reed *et al.*, 1983). Such compartmentation, however, remains to be demonstrated for *S. cerevisiae*.

In summary, our data strongly support a previously undemonstrated role for GSH in *S. cerevisiae*. GSH

might have a storage function, similar to glycogen, trehalose, polyphosphate or lipids (Gancedo & Serrano, 1989). In *S. cerevisiae* γ -GT is a vacuolar enzyme and, in the strain investigated, about 50% of the total cellular GSH is concentrated in the central vacuole (Jaspers & Penninckx, 1984). It is suggested, therefore, that GSH fulfils the role of a storage compound which can be mobilized during starvation and/or reproduction. Substantial portions of the intracellular amino acids are compartmentalized within the cell, mainly in the vacuole. Redistribution of amino acids between compartments in response to metabolic signals has been demonstrated (Wiemken, 1980; Messenguy *et al.*, 1980; Kitamoto *et al.*, 1988). However, to what extent compartmentalization of metabolites may play a role in regulation of pathways other than in amino acid biosynthesis is as yet unknown and further research work along these lines is in progress.

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