Mutations Affecting the Sulphur Assimilation Pathway in *Aspergillus nidulans*: Their Effect on Sulphur Amino Acid Metabolism

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Several *sul-reg* mutants of *Aspergillus nidulans* isolated as constitutive for arylsulphatase were studied with respect to the regulation of enzymes involved in cysteine and homocysteine synthesis and to the pool of sulphur amino acids. All mutants examined showed a decreased concentration of glutathione as compared with the wild type, and all mutants, with one exception, had a decreased total pool of sulphur amino acids. The results suggest that the mutants are leaky in the sulphate assimilation pathway. They show derepression of cysteine synthase, homocysteine synthase, cystathionine β -synthase and γ -cystathionase. In spite of having derepressed homocysteine synthase, the enzyme which constitutes an alternative pathway for homocysteine synthesis, the *sul-reg* mutations do not suppress lesions in genes required for the main homocysteine-synthesizing pathway. This indicates that the derepression of homocysteine synthase is not in itself sufficient for physiological functioning of this enzyme, but seems to depend also on the effectiveness of cysteine synthesis and sulphide formation.

INTRODUCTION

There are two pathways for the synthesis of homocysteine and cysteine in fungi (Fig. 1). It has been shown in *Aspergillus nidulans* that the pathway involving direct synthesis of homocysteine from *O*-acetylhomoserine and sulphide operates only when the main pathway of cysteine synthesis (cysteine synthase, Fig. 1, step 1) is impaired by mutation (Paszewski & Grabski, 1975). In fact, mutants blocked in step (1) show derepression of enzymes of the sulphur assimilation pathway as well as of the three enzymes constituting an alternative pathway (homocysteine synthase, cystathionine β -synthase and γ -cystathionase; Fig. 1, steps 4, 5 and 6, respectively). It was also found that these mutations are pathway suppressors of the 'cystathionine' pathway (cystathionine γ -synthase and β -cystathionase; Fig. 1, steps 2 and 3, respectively) due to the direct synthesis of homocysteine, which serves as a precursor of both cysteine and methionine. We have also found that the mutants *cysB* and *cysC* show a derepression of arylsulphatase activity. This focused our attention on the arylsulphatase constitutive mutants *sul-reg*, previously isolated and characterized by Siddiqui and his co-workers (Siddiqui *et al.*, 1966; Apte *et al.*, 1974; N. K. Singh, personal communication). It was of interest to know if the regulation of other sulphur-related enzymes is also altered, and whether or not these mutations can suppress *methA*, *methB* and *methG* mutations.

Here we report that most, but not all, *sul-reg* mutations are leaky in their sulphate assimilation. They can grow in normal minimal medium, but the mutations cause a permanent sulphur limitation which, in turn, produces changes in the pool of sulphur amino acids as well as enzyme regulation. In fact, these mutants provide a very useful model to study the effects of sulphur limitation without altering the growth medium.

Fig. 1. An outline of sulphur amino acid metabolism in *Aspergillus nidulans*. Enzymes: (1) cysteine synthase, (2) cystathionine γ -synthase, (3) β -cystathionase, (4) homocysteine synthase, (5) cystathionine β -synthase, (6) γ -cystathionase, (7) ATP sulphurylase, (8) sulphite reductase. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; PAPS, adenosine 3'-phosphate 5'-phosphosulphate; APS, adenosine 5'-phosphosulphate.

METHODS

Strains. The following strains of *A. nidulans* were kindly provided by Dr N. K. Singh (Tate Institute of Fundamental Research, Bombay, India): *sul-regA1(nicB8 riboB2)*, *sul-regB2(lysB5 nicB8)*, *sul-regC7(pyroA4)*, *sul-regD11(lysB5 nicB8)* and *sul-regE14(lysB5)*. Strains *mecB10 anA1 phenA2 biA1*, blocked in γ -cystathionase (Pieniżek *et al.*, 1973*a*) and *mecC13(anA1 biA1)*, deficient in *S*-adenosylmethionine synthetase (Pieniżek *et al.*, 1973*b*), were obtained from the collection of the Department of Genetics, University of Warsaw. The strain *cysD1*, impaired in homocysteine synthase, was described previously (Paszewski & Grabski, 1975). The wild type used as reference strain in the experiments was *pyro4 yA1*.

Media, culture conditions and extract preparation. Liquid minimal medium described previously (Paszewski & Grabski, 1974), was supplemented with sulphur amino acids as indicated. Cultures were started by inoculation of 100 ml medium in 300 ml Erlenmeyer flasks with 3–5 ml of a heavy conidial suspension, and grown in a rotary shaker (150 r.p.m.) at 30 °C for 16–18 h. Mycelia were collected on surgical gauze, washed three times with distilled water and blotted on filter paper. For enzyme assays, extracts were prepared from mycelial pads as described previously (Paszewski & Grabski, 1973) with the use of 0.1 M-Tris/HCl buffer, pH 8.0, in the case of ATP-sulphurylase and arylsulphatase determination, and 0.1 M-potassium phosphate buffer, pH 7.5, for the remaining enzymes.

Enzyme assays. β -Cystathionase (EC 4.4.1.8) and γ -cystathionase (EC 4.4.1.1) were assayed as described by Paszewski & Grabski (1974). Homocysteine synthase (EC 4.2.99.10) was assayed in the reaction mixture described previously (Paszewski & Grabski, 1973); the homocysteine formed was determined by the method of Krędić & Tomkins (1966). Cysteine synthase (EC 4.2.99.8) and cystathionine β -synthase (EC 4.2.1.22) were assayed according to Pieniżek *et al.* (1973) and ATP-sulphurylase (EC 2.7.7.4) and sulphite reductase (EC 1.8.1.2) were assayed by the methods of de Vito & Dreyfuss (1964). The sulphide formed was determined according to Siegel (1965). Arylsulphatase (EC 3.1.6.1) was assayed according to Metznerberg & Parson (1966), calculating the activity with the use of a molar extinction coefficient of 12000 for *p*-nitrophenol (Razzell, 1963).

Protein determination. Protein was estimated by the method of Lowry or by the method of Bradford (1976).

DEAE-cellulose chromatography. Cellular extract for this purpose was prepared in 0.05 M-Tris/HCl buffer, pH 7.8, containing 40 M-pyridoxal 5'-phosphate and 1 mM-EDTA. Homogenates were centrifuged at 15000 *g* for 10 min and subsequently at 100000 *g* for 1 h. The supernatant was diluted with extraction buffer to a protein concentration of 1 mg ml⁻¹; 40 ml of such an extract was applied onto the DEAE-cellulose column (1.0 × 15 cm) equilibrated with the same buffer at a flow rate of 20 ml h⁻¹. The column was washed with 40 ml of the extraction buffer and subsequently with 300 ml of a linear salt gradient, 0.0 to 0.3 M-NaCl in the same buffer. The flow rate was adjusted to 10 ml h⁻¹. Fractions of 3 ml were collected and 0.05 or 0.1 ml samples were taken for determination of enzyme activity.

Polyacrylamide gel electrophoresis. Protein separation was carried out according to Laemmli (1970) with the use of Bethesda Research Laboratories preparative gel electrophoresis system (BRL no. 1110PG). Details are given in the legend to Fig. 3.

Determination of pools of ³⁵S-labelled amino acids. The strains were grown in minimal medium supplemented with Na₂³⁵SO₄ (4.8 × 10⁹ c.p.m. mmol⁻¹) for 18 h in standard conditions. The mycelia were harvested and ³⁵S-

labelled amino acids were isolated and separated by thin-layer chromatography on cellulose, as described previously (Paszewski & Grabski, 1974). The position of the radioactive spots was determined by autoradiography. The main band (about 90% of the radioactivity), containing poorly separated glutathione, cystathionine, cystine, homocystine and mixed disulphides, was scraped off the plates. The amino acids were eluted from the cellulose with 0.1 M-HCl. The eluate was evaporated and the residue oxidized with a mixture of H₂O₂ and formic acid, followed by separation of its contents by high-voltage electrophoresis, as described by Bessman *et al.* (1967), together with internal standards of identically oxidized L-cysteine, DL-homocysteine, L-glutathione and L-cystathionine. The positions of particular amino acids were determined by staining with ninhydrin. The spots were cut out and the radioactivity counted in a PPO/POPOP scintillation mixture. ³⁵S-Methionine and ³⁵S-hypotaaurine were estimated directly by scraping their respective spots from the chromatogram and counting the radioactivity.

Genetic techniques. Crossing methods and nutritional tests were as described by Pontecorvo *et al.* (1953). For screening colonies with constitutive arylsulphatase activity, the plates were flooded with 30 mM-indoxyl sulphate in 50 mM-Tris buffer, pH 8.0. Colonies with active enzyme turn blue.

Reagents. L-Cystathionine, L-cysteine, DL-homocysteine thiolactone HCl, L-serine, L-methionine and indoxyl sulphate were obtained from Sigma. Pyridoxal 5'-phosphate was a product of Merck. ³⁵S-labelled sodium sulphate was obtained from the Institute of Nuclear Research, Swierk, Poland. O-Acetyl-L-serine and O-acetyl-L-homoserine were synthesized according to Wiebers & Garner (1967). DEAE-cellulose, type DE52, was obtained from Whatman. N,N'-methylenebisacrylamide and sodium dodecyl sulphate were from BDH and acrylamide from Merck. All inorganic chemicals were of reagent grade.

RESULTS

Levels of sulphur metabolizing enzymes in the wild type and sul-reg strains

The results presented in Table 1 indicate that *sul-reg* mutants isolated as constitutive for arylsulphatase show elevated levels of several enzymes of sulphur metabolism. ATP sulphurylase and sulphite reductase exhibit a similar degree of derepression to that observed in the *cysB1* and *cysC1* mutants, apparently impaired in the last step of cysteine synthesis (Paszewski & Grabski, 1975). There are two exceptions: *sul-regD11* has a low activity of ATP sulphurylase and it may be a leaky mutation in the structural gene coding this enzyme; *sul-regB2* shows a decreased level of sulphite reductase. The levels of these two enzymes are only slightly lower than those observed in the wild type, but it is very likely that the mutants produce more of the respective enzymes but with lower specific activities. Therefore, the levels of these two enzymes should be compared with those observed in other *sul-reg* mutants rather than with the wild type. Additionally, the *sul-regB2* strain, unlike *sul-regD1*, shows a decreased level of β -cystathionase. The three enzymes constituting the alternative pathway of homocysteine and cysteine synthesis, i.e. homocysteine synthase, cystathionine β -synthase and γ -cystathionase, show a similar degree of derepression as observed previously in the *cysB1* and *cysC1* mutants. The activity of cysteine synthase is 2 to 3-fold higher in the *sul-reg* strains as compared with the wild type. It was observed in *Saccharomyces cerevisiae* (Yamagata *et al.*, 1976), *Cephalosporium acremonium* (Dobeli & Nuesch, 1980) and *Saccharomycopsis lipolytica* (Morzycka & Paszewski, 1982) that homocysteine synthase also exhibits cysteine synthase activity. If this is also the case for *A. nidulans*, the elevated level of cysteine synthase activity in *sul-reg* strains could be attributed to homocysteine synthase. To check this, the *cysD1* mutation (impaired homocysteine synthase) was introduced into the *sul-regD11* strain. As shown in Table 2, the double mutant *sul-regD11 cysD1* exhibits about a 3-fold decrease of cysteine synthase activity and about a 12-fold decrease of homocysteine synthase activity, as compared with the *sul-regD11* mutant. Data presented in Fig. 2 clearly indicate that there are two enzyme fractions with cysteine synthase activity, one of which also exhibits homocysteine synthase activity. The fact that the second cysteine synthase peak and the homocysteine synthase peak both disappeared in the extract of the double mutant suggests the presence of a bifunctional (at least *in vitro*) enzyme which is derepressed in *sul-reg* strains and impaired in strains carrying the *cysD1* mutation. (We were able to detect some activity of the bifunctional enzyme in the double mutant by washing the DEAE-cellulose column with 0.07 M-NaCl.) The two activities co-migrate on polyacrylamide gel electrophoresis (Fig. 3), supporting the hypothesis that a single enzyme is involved. The *cysD1* mutation does not affect the regulation of other enzymes which are derepressed in *sul-reg* strains. As shown in

Table 1. Activities of enzymes of *S*-amino acid biosynthesis in the wild-type and *sul-reg* strains

The unit of activity is nmol min⁻¹ (mg protein)⁻¹. The results represent the means of 3-6 independent experiments $\pm$ SEM.

Strain	ATP-sulphurylase	Sulphite reductase	Cysteine synthase	Homocysteine synthase	β -Cystathionase	γ -Cystathionase	Cystathionine β -synthase
Wild type	51.3 $\pm$ 15.3	0.53 $\pm$ 0.28	2.73 $\pm$ 0.72	69.9 $\pm$ 18.4	2.60 $\pm$ 0.77	0.51 $\pm$ 0.2	0.57 $\pm$ 0.25
<i>sul-regA1</i>	86.6 $\pm$ 19.8	0.94 $\pm$ 0.27	8.87 $\pm$ 1.88	281.0 $\pm$ 69.2	5.33 $\pm$ 1.7	1.21 $\pm$ 0.44	1.42 $\pm$ 0.3
<i>sul-regB2</i>	87.9 $\pm$ 19.8	0.36 $\pm$ 0.01	7.29 $\pm$ 1.2	254.5 $\pm$ 70.1	1.41 $\pm$ 0.57	0.92 $\pm$ 0.45	1.30 $\pm$ 0.25
<i>sul-regC7</i>	64.2 $\pm$ 22.3	1.40 $\pm$ 0.27	4.54 $\pm$ 1.06	256.1 $\pm$ 60.2	5.00 $\pm$ 1.56	0.85 $\pm$ 0.10	0.74 $\pm$ 0.11
<i>sul-regD11</i>	19.4 $\pm$ 1.5	1.11 $\pm$ 0.46	10.05 $\pm$ 1.75	218.1 $\pm$ 55.7	3.72 $\pm$ 1.1	1.49 $\pm$ 0.80	1.72 $\pm$ 0.17
<i>sul-regE14</i>	84.8 $\pm$ 9.3	1.25 $\pm$ 0.73	5.00 $\pm$ 0.47	332.9 $\pm$ 27.8	2.61 $\pm$ 1.0	1.28 $\pm$ 0.20	1.33 $\pm$ 0.38

Table 2. Effect of *mecB*, *mecC* and *cysD* mutations on cysteine synthase and homocysteine synthase activities in *sul-reg* strains

The unit of activity is nmol min⁻¹ (mg protein)⁻¹. Where indicated, minimal medium (MM) was supplemented with L-cysteine (3 mM), DL-homocysteine thiolactone (4 mM) or L-methionine (3 mM). The results represent the means of 3 independent experiments $\pm$ SEM. —, Not estimated.

Strain	Cysteine synthase			Homocysteine synthase			Arylsulphatase	
	MM	MM +		MM	MM +		MM	MM
		cysteine	homocysteine		cysteine	homocysteine		
<i>sul-regD11</i>	9.48 $\pm$ 1.4	5.23 $\pm$ 0.9	5.20 $\pm$ 0.7	243.3 $\pm$ 47.2	106.3 $\pm$ 8.5	59.8 $\pm$ 34.5	57.0 $\pm$ 16.6	121.2 $\pm$ 21.2
<i>mecB10</i>	4.06 $\pm$ 1.4	4.41 $\pm$ 0.2	—	45.6 $\pm$ 3.5	51.5 $\pm$ 2.0	—	—	—
<i>mecB10 sul-regD11</i>	10.10 $\pm$ 4.8	5.06 $\pm$ 2.1	4.50 $\pm$ 0.8	263.5 $\pm$ 96.0	84.4 $\pm$ 36.4	68.7 $\pm$ 22.6	—	—
<i>mecC13</i>	2.2 $\pm$ 0.12	—	—	83.0 $\pm$ 31.4	—	—	54.0 $\pm$ 9.95	—
<i>mecC13 sul-regD11</i>	10.87 $\pm$ 2.2	—	4.52 $\pm$ 1.9	300.0 $\pm$ 42.2	—	94.7 $\pm$ 13.1	217.9 $\pm$ 90.7	—
<i>cysD1</i>	3.37 $\pm$ 0.6	—	—	21.2 $\pm$ 4.2	—	—	—	4.8 $\pm$ 1.7
<i>cysD1 sul-regD11</i>	3.71 $\pm$ 0.6	—	—	20.3 $\pm$ 10.0	—	—	—	107.0 $\pm$ 23.8

Fig. 2. DEAE-cellulose elution profile of cysteine synthase and homocysteine synthase from *A. nidulans*. Monofunctional cysteine synthase elutes in the flow-through and bifunctional cysteine-homocysteine synthase elutes with about 0.07 M-NaCl. $\circ$, Cysteine synthase; Δ , homocysteine synthase; ----, protein. (a) Wild type, (b) *sul-regD₅*, (c) *cysD1 sul-regD11₅*. The results are from a single representative experiment.

Table 2, the double mutant *sul-regD11 cysD1* has a high level of arylsulphatase, corresponding to that of the single *sul-reg* mutant. It is likely, therefore, that *cysD1* is a structural locus coding for the bifunctional enzyme.

Cysteine synthase in the *sul-reg* mutants is repressible under conditions of homocysteine synthase repression. As shown in Table 2, methionine, homocysteine and cysteine cause repression of both synthases when supplemented in the growth medium. The fact that repression of homocysteine synthase is stronger than that of cysteine synthase suggests that it is mainly, if not only, the bifunctional enzyme which is repressed. Cysteine synthase and homocysteine synthase were also found to be repressible in the remaining four *sul-reg* mutants. It seems that the enzyme derepression observed in these strains is unlikely to result from a mutation in regulatory genes, but rather is due to an alteration in the pool of sulphur amino acids. The *mecC13* mutation (a partial loss of *S*-adenosylmethionine synthetase) protects the enzymes against repression by methionine but not by homocysteine, indicating that methionine must be metabolized to homocysteine to cause enzyme repression. The latter compound causes repression even under conditions when *mecB1* mutation (a lesion in γ -cystathionase) prevents its conversion to cysteine.

In extracts of the *sul-regD11 cysD1* strain we observed a higher level of monofunctional cysteine synthase than in the wild type. This may explain the fact that the total activity of the cysteine synthase in the double mutant is not lower than in the wild type, despite the drastic reduction of bifunctional enzyme activity. It is possible that there is some regulatory relationship between these two enzymes, but this problem requires further investigation.

Fig. 3. Polyacrylamide gel electrophoresis of homocysteine synthase from *A. nidulans*. A sample (1 ml) containing 3.5 mg protein from the peak fractions of homocysteine synthase activity from the DEAE-cellulose column was applied to a 10% (w/v) separating gel (1 cm diameter, 4.5 cm height). Electrophoresis was started with 2 mA current followed by 6 mA after the whole sample had entered the gel. Outcoming proteins were eluted continuously with the electrode buffer at the rate of 12 ml h⁻¹. Fractions (0.9 ml) were collected; 0.05 or 0.1 ml of each fraction was withdrawn for enzyme determination. Δ , Homocysteine synthase; $\circ$, cysteine synthase; ----, protein.

Table 3. Accumulation of ³⁵S-labelled amino acids in mycelia of the wild type and *sul-reg* mutants grown in the presence of ³⁵SO₄²⁻

Mycelia were grown in minimal medium containing ³⁵SO₄²⁻ (4.5 × 10⁸ c.p.m. mmol⁻¹) for 18 h at 30 °C. The data are from a single experiment. Similar ratios were obtained in three analogous experiments. The results are expressed as 10⁻⁵ × c.p.m. per g dry wt.

Strain	Cysteine*	Homocysteine*	Glutathione*	Cystathionine†	Methionine	Total‡
Wild type	20.4	16.5	206.2	38.3	33.7	352.6
<i>sul-regA1</i>	13.3	10.2	150.1	48.2	48.8	424.0
<i>sul-regB2</i>	12.7	10.3	54.9	35.9	21.1	209.4
<i>sul-regC7</i>	12.1	13.5	85.6	30.2	34.8	234.1
<i>sul-regD11</i>	7.2	5.3	114.8	40.3	39.3	304.3
<i>sul-regE14</i>	12.8	9.3	64.6	56.2	31.9	220.7

* Estimated as sulphonic acids. (It was not estimated what fraction of these amino acids was originally in the thio- and what fraction was in the disulphide form.)

† Estimated as cystathionine sulphone.

‡ Total ³⁵S retained on Dowex 50 (H⁺).

The pools of sulphur amino acids in the sul-reg mutants differ from those found in the wild type

The pools were studied by growing the strains in the presence of Na₂³⁵SO₄ and measuring the radioactivity of several sulphur amino acids found in the water extracts from mycelia. The results of a representative experiment are given in Table 3. While the differences in the ratio of cysteine and homocysteine between the mutants and the wild type were not always significant, the differences in glutathione and the total amount of ³⁵S were very reproducible. Even the *sul-regA1* strain which shows an increased total ³⁵S pool has less glutathione compared to the wild type. The remaining *sul-reg* strains contain less sulphur in the pool; this is probably the result of some restrictions in the sulphate assimilation pathway. Enzyme lesions in this pathway were

Table 4. Results of crosses between the *cysB1*, *methB3* strain and various *sul-reg* strains

Cross	No. of progeny tested	No. of Meth ⁻ segregants		
		Total	With <i>sul-reg</i> mutation	Without <i>sul-reg</i> mutation
<i>cysB1 methB3</i>				
× <i>sul-regA1</i>	130	37	21	16
× <i>sul-regB2</i>	148	30	15	15
× <i>sul-regC7</i>	126	35	22	13
× <i>sul-regD11</i>	150	38	17	21
× <i>sul-regE14</i>	149	31	16	15

found in the *sul-regB2* and *sul-regD11* strains but not all enzyme activities in this pathway were assayed, so it is possible that the remaining mutants are impaired in other steps.

sul-reg mutations do not suppress lesions in the cysteine to homocysteine pathway

Strain *cysB1 methB3* (*cysB1* suppresses the *methB3* lesion) was crossed with all *sul-reg* strains to determine whether these mutations suppress the *methB3* mutation, and if so, whether they are allelic with *cysB*. The results, shown in Table 4, indicate that the answer to both questions is negative, i.e. in all crosses about one quarter of progeny required methionine for growth, and about one half of Meth⁻ segregants contained the *sul-reg* marker. Similar results were obtained in crosses between *sul-reg* mutants and *methG55* strain. The *sul-reg* mutations do not suppress the *methB* and *methG* lesions. It was pointless, therefore, to test the allelism of the *sul-reg* mutations used in this study with other methionine suppressors: *cysA1*, *cysC1* and *suAmeth* (Paszewski & Grabski, 1975).

DISCUSSION

The results obtained in this study indicate that the derepression in *sul-reg* mutants of a number of enzymes involved in the synthesis of sulphur amino acids results from sulphur limitation caused by a partial impairment of the sulphate assimilation pathway. This conclusion is strongly supported by the fact that a number of other *sul-reg* mutants, allelic to those studied here, require a source of reduced sulphur for growth, or are stimulated by sulphite or methionine (Apte *et al.*, 1974). Interestingly, some *sul-reg* mutants have been found to be allelic to mutants blocked in the sulphate assimilation pathway (*s* loci) described by Arst (1968) (N. K. Singh, personal communication). It is evident that the enzyme derepression correlates best with a diminution of the glutathione pool among the sulphur compounds examined. A similar situation was found in *Saccharomycopsis lipolytica* (Morzycka *et al.*, 1976). It is possible that glutathione, in addition to cysteine, may act as a regulatory effector.

The physiological role of homocysteine synthase in *A. nidulans* is not clear. The enzyme is not essential for the wild type but is necessary for the *cysA*, *cysB*, *cysC* mutants blocked in the main pathway of cysteine synthesis (Pieniżek *et al.*, 1974; Paszewski & Grabski, 1975), since double mutants *cysA1 cysD1*, *cysB1 cysD1* and *cysC1 cysD1* are auxotrophic. When mycelia of these double mutants are transferred for several hours from a methionine-containing medium to a minimal medium with ³⁵SO₄²⁻ as the sole sulphur source, only traces of radioactive sulphur can be detected in the pool of amino acids.

We have demonstrated that homocysteine synthase is necessary for the recycling of the methylthio group of 5'-methylthioadenosine to methionine (Guranowski & Paszewski, 1982), but again this recycling does not seem necessary for the growth of the normal strain. Also, the cysteine synthase activity of this enzyme observed *in vitro* does not seem to operate *in vivo* because the double mutants *cysB1 mecB10* or *cysB1 mecA1* are auxotrophic (Paszewski & Grabski, 1973). There is evidence suggesting that a corresponding bifunctional enzyme in *Saccharomyces cerevisiae* (Yamagata *et al.*, 1975) and in *Saccharomycopsis lipolytica* (Morzycka & Paszewski, 1982) expresses the two activities both *in vitro* and *in vivo*.

Our data indicate that the monofunctional cysteine synthase is also subject to regulation. Its

activity is clearly elevated in the double mutant *sul-regD11 cysDI*, that is under conditions of sulphide limitation and impairment of the alternative pathway of cysteine synthesis.

The *sul-reg* mutations, in spite of causing a derepression of homocysteine synthase, similar to or even higher than that observed in *cysB1* and *cysC1* mutants, do not suppress mutations in the *methA*, *methB* and *methG* genes specifying the cysteine to homocysteine pathway (steps 2 and 3, Fig. 1). Therefore, a derepression of homocysteine synthase is not sufficient for suppression. The *cysB1* and *cysC1* mutants are blocked in the last step of cysteine synthesis, but not in the sulphur assimilation pathway. In these mutants this pathway is derepressed and thus provides more sulphide than in the wild type. Sulphide can be utilized for homocysteine synthesis in the reaction catalysed by homocysteine synthase. Additionally, a shortage of cysteine makes the second substrate, *O*-acetylhomoserine, which is normally utilized for cystathionine synthesis, available for direct formation of homocysteine. In the *sul-reg* mutants, sulphide may be a limiting substrate and predominantly used for cysteine synthesis. This is presumably the cause of the failure of *sul-reg* mutations to suppress lesions in *meth* mutants. Since this is true in the case of *methB* mutants blocked in cystathionine β -synthase, the availability of *O*-acetylhomoserine for homocysteine synthesis does not seem to be a crucial factor determining the suppression.

One might expect, therefore, that all mutants which are derepressed in the sulphate assimilation pathway, and thus able to produce more sulphide, should suppress methionine mutations, even without elevation of the homocysteine synthase level.

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