Physiological analysis of mutants of *Saccharomyces cerevisiae* impaired in sulphate assimilation

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The assimilation of sulphate in *Saccharomyces cerevisiae*, comprising the reduction of sulphate to sulphide and the incorporation of the sulphur atom into a four-carbon chain, requires the integrity of 13 different genes. To date, the functions of nine of these genes are still not clearly established. A set of strains, each bearing a mutation in one *MET* gene, was studied. Phenotypic studies and enzyme determinations showed that the products of at least five genes are needed for the synthesis of an enzymically active sulphite reductase. These genes are *MET1*, *MET5*, *MET8*, *MET10* and *MET20*. Wild-type strains of *S. cerevisiae* can use organic metabolites such as homocysteine, cysteine, methionine and *S*-adenosylmethionine as sulphur sources. They are also able to use inorganic sulphur sources such as sulphate, sulphite, sulphide or thiosulphate. Here we show that both of the two sulphur atoms of thiosulphate are used by *S. cerevisiae*. Thiosulphate is cleaved into sulphite and sulphide prior to utilization by the sulphate assimilation pathway, as the metabolism of one sulphur atom from thiosulphate requires the presence of an active sulphite reductase.

Introduction

In the yeast *Saccharomyces cerevisiae*, the assimilation of sulphate calls into play a set of five enzymic reactions, in addition to its uptake (Fig. 1). Sulphate reduction requires first an initial 'activation' to phosphosulphate compounds. As in *Escherichia coli* and *Salmonella typhimurium*, this activation is a two-step process in *S. cerevisiae*: ATP sulphurylase (encoded by the *MET3* gene; Cherest *et al.*, 1987) first catalyses the reaction of ATP and sulphate to give adenosine 5'-phosphosulphate (APS); then APS kinase (encoded by the *MET14* gene, Korch *et al.*, 1991) carries out a phosphorylation step that leads to the formation of 3'-phosphoadenosine 5'-phosphosulphate (PAPS). The reduction of the sulphur atom is then achieved through two enzymic reactions. The first one, catalysed by PAPS reductase (encoded by the *MET16* gene, Thomas *et al.*, 1990) yields sulphite. Recent results (Thomas *et al.*, 1990) have given new support to the first hypothesis of Tsang & Schiff (1976) which involves the transfer of the sulphonyl moiety of PAPS to a thiol acceptor, presumably as a bound S-SO₃ form. Then, sulphite reductase, a complex enzyme

containing both a sirohaem and an iron-sulphur cluster, carries out the six-electron reduction of sulphite to sulphide. It is now clear that in yeast, sulphide is incorporated into a four-carbon chain yielding homocysteine (Cherest & Surdin-Kerjan, 1992). Homocysteine synthase, which catalyses the synthesis of homocysteine from *O*-acetylhomoserine and sulphide, is encoded by the *MET25* gene (Kerjan *et al.*, 1986; Fig. 1).

In *S. cerevisiae*, methionine and cysteine both derive their sulphur from homocysteine while, in eubacteria, the sulphur atom of methionine is derived from cysteine (Cohen & Saint Girons, 1987). Furthermore, eubacteria like *E. coli* cannot use methionine as sole sulphur source since they do not possess cystathionine β -synthase and γ -cystathionase activities. By contrast, the presence of the two trans-sulphuration pathways in *S. cerevisiae* allows it to use both methionine and cysteine as effective sulphur sources.

In addition to the genes *MET3*, *MET14*, *MET16* and *MET25* cited above, the products of nine other genes, *MET1*, *MET4*, *MET5*, *MET8*, *MET10*, *MET18*, *MET19*, *MET20* and *MET22*, have been implicated in sulphate assimilation in yeast without their functions being clearly established (Masselot & de Robichon-Szulmajster, 1975). Recently, the roles of two of these genes were deciphered: *MET4* was shown to encode the transcriptional activator of this pathway (Thomas *et al.*,

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Abbreviations: APS, adenosine 5'-phosphosulphate; PAPS, 3'-phosphoadenosine 5'-phosphosulphate.

Fig. 1. Metabolism of sulphur amino acids in *S. cerevisiae*.

1992) while, unexpectedly, *MET19* was proved to be the structural gene of yeast glucose-6-phosphate dehydrogenase (Thomas *et al.*, 1991). The reasons why mutation or inactivation of the gene encoding the first enzyme of the pentose phosphate pathway leads to methionine auxotrophy are not yet understood.

The present work aims to clarify both phenotypic and enzymic characteristics of strains mutated in those genes involved in sulphate assimilation, the functions of which are still unknown.

Methods

Strains. The *S. cerevisiae* strains used are listed in Table 1. They are all derived from the parental strain CC359-OL2.

Media. YPG, YNBG and the sulphur-free medium B were as described in Cherest & Surdin-Kerjan (1992). Cells grown in complete YPG medium were centrifuged and washed twice before being used to inoculate B or YNBG medium. The BIG-YPG medium was modified from Rikkerrink *et al.* (1988). It contained (w/v): 1% glucose, 1% β -alanine, 0.25% yeast extract, 1% peptone, 0.1% bismuth ammonium citrate and 0.3% sulphite. With thiosulphate, BIG-YNB medium was used. It contained 1% glucose, 0.7% Yeast Nitrogen Base (Difco), 0.1% bismuth ammonium citrate and 50 mM-thiosulphate, and was supplemented to meet the auxotrophic requirements of the strains. The bismuth-containing media were filter-sterilized.

Cell growth and preparation of cell-free extracts. For enzyme determinations, cells were grown in YNBG medium supplemented to

Table 1. *Strains used*

Strain	Genotype	Source*
CC359-OL2	<i>MATα his3 leu2 ura3</i>	Cherest <i>et al.</i> (1985)
CC346-3B	<i>MATα ura3 met3</i>	H. Cherest
CC366-9C	<i>MATα his3 leu2 ura3 met14</i>	H. Cherest
CC362-2A	<i>MATα leu2 ura3 met16</i>	Thomas <i>et al.</i> (1990)
C112	<i>MATα his3 ura3 met25::HIS3</i>	Thomas <i>et al.</i> (1989)
CC469-13	<i>MATα ura3 met1</i>	H. Cherest
CC361-1B	<i>MATα ura3 met5</i>	H. Cherest
CC496-43	<i>MATα his3 ura3 met8</i>	Cherest <i>et al.</i> (1990)
CC501-2	<i>MATα leu2 ura3 met10</i>	H. Cherest
CC363-20B	<i>MATα leu2 ura3 met18</i>	H. Cherest
CC370-8C	<i>MATα ura3 met20</i>	H. Cherest
CC471-1D	<i>MATα leu2 ura3 met4</i>	Thomas <i>et al.</i> (1992)
CC364-18C	<i>MATα his3 ura3 met22</i>	H. Cherest

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meet the auxotrophic requirements of the strains. For homocysteine synthase and ATP sulphurylase determinations, cells from 50 ml cultures were collected by centrifugation, then washed and resuspended in 500 μ l of buffer A (100 mM-Tris/HCl, pH 7.5, 0.1 mM-EDTA, 10% w/v, glycerol, 1 mM-phenylmethylsulphonyl fluoride). Glass beads (500 μ l, 0.45 mm diam.) were added and the extraction was performed as described in Thomas *et al.* (1989). The extract was dialysed for 16 h against 20 mM-Tris/HCl, pH 7.5, containing 0.1 mM-EDTA and 10% glycerol. For PAPS reductase and sulphite reductase assays, cells from 200 ml cultures were collected by centrifugation, then washed and suspended in 800 μ l of buffer A containing 1 mM-2-mercaptoethanol. The cell suspension was separated into two micro-

centrifuge tubes and 500 µl of glass beads was added to each tube. The extraction was performed also as described in Thomas *et al.* (1989). Sulphite reductase was assayed on the crude extracts and PAPS reductase was assayed after dialysis (see below).

Enzyme assays. Homocysteine synthase and ATP sulphurylase were assayed as described in Thomas *et al.* (1992). Sulphite reductase was assayed by the method of de Vito & Dreyfuss (1964) and the sulphide formed was estimated by the method of Siegel (1965). Four protein concentrations were assayed for each extract and were incubated for 10 min at 37 °C in the assay medium prior to addition of the substrate (sulphite). As noted in the text, an improved assay for PAPS reductase activity was used: extracts were first dialysed at 4 °C for 4 h against four changes of 20 mM-Tris/HCl, pH 7.5 containing 1 mM-2-mercaptoethanol, 0.1 mM-EDTA, 5 mM-dithiothreitol and 10% glycerol. Four protein concentrations were used for each extract. The assay medium contained: 50 mM-Tris/HCl pH 7.5, 5 mM-dithiothreitol and 20, 30, 40 and 50 µl of cell-free extract in a final volume of 90 µl. After a pre-incubation at 37 °C for 10 min, 10 µl of a 5 mM solution of PAPS was added and the incubation was continued for 60 min at 37 °C. In this reaction, endogenous thioredoxin is reduced in a purely chemical fashion by dithiothreitol (Schwenn *et al.*, 1988). For each assay, a control without PAPS was run. The sulphite formed in the reaction was estimated by the method of Grant (1947). A slight activation of PAPS reductase activity was measured at higher protein concentrations.

Protein concentrations were estimated by the Lowry method.

Intracellular sulphite concentration. Strains to be tested were grown in 10 ml of minimal medium YNBG supplemented according to the auxotrophic requirements of the different strains. When the cellular concentration was about 10^7 cells ml⁻¹, cells were collected by centrifugation and suspended in 250 µl of water. Chloroform (15 µl) and 0.1% SDS (5 µl) were added to the cell suspension. Vortexing at maximum speed for 30 s followed by a 5 min incubation at 30 °C released soluble metabolites from the cells. The permeabilized cells were removed by centrifugation and the sulphite contained in the supernatant was estimated on 25 and 50 µl samples by the method of Grant (1947).

Reproducibility. Except where otherwise stated, the figures given in the Tables are mean values from at least two experiments. In each experiment, the deviation from this mean value was at most 10%.

Results

Growth rate of methionine auxotrophic strains on different sulphur sources

The phenotypic study of strains mutated in genes *MET1*, *MET5*, *MET8*, *MET10*, *MET18*, *MET20* and *MET22* was initiated by measuring the growth rates of each mutant in the presence of various inorganic and organic sulphur sources. The sulphur compounds tested were added to the sulphur-free medium B. Strains impaired in known functions as well as the parental strain were used as controls. As expected, organic sulphur sources (i.e. homocysteine, cysteine, methionine and S-adenosylmethionine) satisfied the sulphur requirement of all mutants (Table 2). In the case of inorganic sulphur sources, sulphate supported the growth of the wild-type

Table 2. Growth of methionine auxotrophic mutants on different sulphur sources

The mean generation time was calculated from growth curves. One growth-curve experiment was performed for each strain in each medium. —, No growth; +, growth for at least five generations.

Strain	Relevant genotype	Mean generation time (h)						
		SO ₄ ²⁻	SO ₃ ²⁻	S ²⁻	HC	Met	Cys	SAM
CC359-OL2	—	3.5	3.5	+	3.5	3.0	4.5	3.0
CC346-3B	<i>met3</i>	—	3	+	3	2.75	5.0	3.5
CC366-9C	<i>met14</i>	—	4.75	+	4.0	4.0	3.5	3.0
CC362-2A	<i>met16</i>	—	5.0	+	5.5	5.0	5.0	3.5
CC365-1A	<i>met25</i>	—	—	—	3.0	3.0	7.5	3.5
CC469-13	<i>met1</i>	—	—	+	3.0	3.0	3.5	3.0
CC361-1B	<i>met5</i>	—	—	+	3.5	2.5	7.5	3.0
CC469-43	<i>met8</i>	—	—	+	3.0	3.5	4.5	4.0
CC501-2	<i>met10</i>	—	—	+	3.5	3.0	3.0	3.0
CC363-7A	<i>met18</i>	—	—	+	3.0	3.0	6.5	3.5
CC370-8C	<i>met20</i>	—	—	+	4.0	3.5	5.5	2.5
CC471-1D	<i>met4</i>	—	—	—	2.5	3.0	6.0	2.5
CC364-18C	<i>met22</i>	—	—	—	4.5	3.0	5.5	5.5

* The different sulphur sources, added to sulphur-free B medium, were: SO₄²⁻, sulphate (0.5 mM); SO₃²⁻, sulphite (0.5 mM); S²⁻, sulphide (0.5 mM); HC, DL-homocysteine (0.2 mM); Met, L-methionine (0.1 mM); Cys, L-cysteine (0.5 mM); SAM, S-adenosylmethionine (0.05 mM).

strain only, while sulphite satisfied the sulphur requirement of the *met3*, *met14* and *met16* mutants. These results are in accord with the functions assigned to these genes since their products catalyse the three steps preceding sulphite synthesis. Growth on sulphide was poor, even for wild-type strains, and did not allow the determination of a precise mean generation time. However, significant growth (for at least five generations) was observed in the case of the *met1*, *met5*, *met8*, *met10*, *met18* and *met20* mutants and, as expected, for the *met3*, *met14* and *met16* mutants. By contrast the *met4*, *met22* and *met25* mutants were absolutely unable to divide in the presence of sulphide as sulphur source.

Intracellular sulphite concentration in different mutants

Study of the gene *MET16* has revealed that it encodes PAPS reductase. This enzyme has been shown to be homodimeric (Schwenn *et al.*, 1988). Northern blot experiments revealed that *MET16* was transcribed in *met1* and *met8* mutants although no PAPS reductase activity was measured in cell-free extracts of these strains (Thomas *et al.*, 1990). To establish if this absence of activity *in vitro* reflected the *in vivo* situation, an assay (described in Methods) that allows determination of the intracellular concentration of sulphite in strains grown in

Table 3. Intracellular sulphite concentration in different methionine auxotrophs

Each figure represents the mean value from at least two independent experiments. The deviation from this mean value was at most 10%. —, Below detection limit.

Strain	Relevant genotype	Intracellular sulphite concentration [nmol sulphite (mg dry wt) ⁻¹]
CC359-OL2	—	—
CC371-1D	<i>met3</i>	—
CC366-9C	<i>met14</i>	—
CC362-2A	<i>met16</i>	—
C112	<i>met25</i>	—
CC471-1D	<i>met4</i>	—
CC364-18C	<i>met22</i>	—
CC469-13	<i>met1</i>	73
CC361-1B	<i>met5</i>	26
CC469-43	<i>met8</i>	83
CC501-2	<i>met10</i>	71
CC363-7A	<i>met18</i>	34
CC370-8C	<i>met20</i>	25

a sulphate-containing medium was devised. Analysis of the results (Table 3) showed that sulphite is not accumulated in three classes of strains: (i) wild-type strains that convert sulphite into sulphide through the metabolic route to sulphur amino acids; (ii) strains that are impaired in an enzyme step situated upstream of sulphite formation, as is the case in strains mutated in genes *MET3*, *MET14* and *MET16*; and (iii) three other mutants namely the *met4* mutant impaired in the transcriptional activation of all genes implicated in sulphate reduction, the *met25* mutant that accumulates sulphide as expected (see below) and the *met22* mutant.

In contrast, *met1*, *met5*, *met8*, *met10*, *met18* and *met20* mutants accumulated sulphite, suggesting that all the corresponding mutations lead to an enzymically inactive sulphite reductase. This experiment also reveals that the *met1* and *met8* mutations do not impair the PAPS reductase activity that is required for sulphite formation.

Reduction of sulphite *in vivo*

In order to establish that the accumulation of sulphite measured in the *met1*, *met5*, *met8*, *met10*, *met18* and *met20* mutants results from a defect in sulphide formation, we performed a complementary test by plating these mutants on sulphite-containing BIG-YPG medium plates. This medium allowed the direct visualization of the sulphide formed *in vivo* by its precipitation into brown bismuth sulphide. As can be seen in Fig. 2(a), *met1*, *met5*, *met8*, *met10*, and *met20* mutants were white when grown on this medium, showing that they do not transform sulphite into sulphide. However, the *met3*,

met16, *met25*, *met4* and *met22* mutants, as well as the parental strain, were brown when grown on this medium, showing that they are able to reduce sulphite to sulphide. These results are in agreement with the sulphite accumulation studies. As expected, the *met25*-disrupted strain turned dark brown due to its deficiency in sulphide incorporation. Unexpectedly, the *met18* mutant was pale brown in this test, although we found that it accumulated sulphite (Table 3).

Enzyme activities *in vitro*

The results described above for the *met1* and *met8* mutations conflict with two preceding papers (Massetot & Surdin-Kerjan, 1977; Thomas *et al.*, 1990) that reported a lack of PAPS reductase activity in cell-free extracts from *met1* and *met8* mutated strains. We modified our procedure for the PAPS reductase assay (see Methods). Using this assay, we detected a measurable PAPS reductase activity in cell-free extracts from all strains except *met16* and *met4* mutants (Table 4). We also determined the ATP sulphurylase, sulphite reductase and homocysteine synthase activities in all the mutated strains, and found that *met1*, *met5*, *met8*, *met10*, and *met20* mutants were devoid of detectable sulphite reductase activity, confirming the results of the physiological tests reported above, as well as those previously reported (Massetot & Surdin-Kerjan, 1977). As expected, the *met3* mutant lacked ATP sulphurylase activity and the *met25* mutant was devoid of homocysteine synthase activity. The *met18* mutant showed no sulphite reductase activity *in vitro*, in accord with the accumulation of sulphite by this mutant but not with its low formation of sulphide in the bismuth test. The *met22* mutant exhibited all enzyme activities. The inability of the *met22* mutant to use sulphate, sulphite and sulphur as sulphur sources thus cannot be explained by an impairment in some enzyme activity involved in sulphate reduction.

Utilization of thiosulphate

To try to understand how *S. cerevisiae* uses thiosulphate, we tested the *met* mutants on thiosulphate-containing BIG-YNB medium. In this case (Fig. 2 b), all the strains turned brown, the *met25*-disrupted strain becoming almost black. This clearly shows that sulphite reductase activity is not required for the synthesis of sulphide from thiosulphate, as strains devoid of sulphite reductase activity are able to carry out this reaction. In other words, yeast contains an enzyme system capable of cleaving thiosulphate to yield sulphide, and the *met25* and *met4* mutants can cleave thiosulphate to yield sulphide. Nevertheless, they are unable to use thiosulphate as a sulphur source (Table 5)

Fig. 2. (a) Physiological characterization of sulphide formation from sulphite in different *met* mutants. The different strains were grown on a minimal medium containing all factors necessary to meet growth requirements. They were then tested on YPG (left) and BIG-YPG medium containing sulphite (right). (b) Physiological characterization of sulphide formation from thiosulphate in different *met* mutants. Strains were tested on BIG-YNB medium containing all factors necessary to meet growth requirements of the strains, together with 50 mM-thiosulphate. The strains were tested in the same order as in (a).

Table 4. *Enzyme activities in different methionine auxotrophs*

Each figure represents the mean value from at least two independent experiments. —, Below detection limit.

Strain	Relevant genotype	Enzyme activity [nmol substrate transformed min ⁻¹ (mg protein) ⁻¹]			
		ATP sulphurylase	PAPS reductase	Sulphite reductase	Homocysteine synthase
CC359-OL2	—	135	0.30	1.4	464
CC346-3B	<i>met3</i>	—	0.32	4.6	422
CC362-2A	<i>met16</i>	373	—	7.4	463
C112	<i>met25</i>	248	0.48	4.3	—
CC469-13	<i>met1</i>	298	0.41	—	803
CC361-1B	<i>met5</i>	238	0.41	—	246
CC496-43	<i>met8</i>	149	0.44	—	674
CC501-2	<i>met10</i>	255	0.33	—	382
CC363-20B	<i>met18</i>	321	0.31	—	213
CC370-8C	<i>met20</i>	487	0.66	—	80
CC471-1D	<i>met4</i>	1.6	—	0.2	37
CC364-18C	<i>met22</i>	155	0.53	5.6	528

Table 5. *Growth of different mutants on thiosulphate*

The growth yield data represent the maximal number of cells obtained in each experiment. Each figure represents the mean value obtained from two experiments. The deviation from this mean value was at most 20%. ND, Not determined.

Strain	Relevant genotype	Growth on thiosulphate (0.25 mM)	Growth yield (mg dry wt ml ⁻¹)	
			Methionine (0.005 mM)	Thiosulphate (0.005 mM)
CC359-OL2	—	+	0.131	0.206
CC346-3B	<i>met3</i>	+	ND	ND
CC362-2A	<i>met16</i>	+	ND	ND
C112	<i>met25</i>	—	ND	ND
CC469-13	<i>met1</i>	+	ND	ND
CC361-1B	<i>met5</i>	+	0.093	0.085
CC496-43	<i>met8</i>	+	ND	ND
CC501-2	<i>met10</i>	+	0.115	0.087
CC363-20B	<i>met18</i>	+	ND	ND
CC370-8C	<i>met20</i>	+	0.075	0.086
CC471-1D	<i>met4</i>	—	ND	ND
CC364-18C	<i>met22</i>	+	ND	ND

Another experiment was performed to determine if both of the sulphur atoms of thiosulphate are used by yeast. In this experiment, it was first determined that 0.005 mM-methionine limited the growth yield of the parental strain CC359-OL2 in B medium. Then strain CC359-OL2 and *met5*, *met10* and *met20* mutants were grown in B medium containing 0.005 mM-methionine or 0.005 mM-thiosulphate. Results (Table 5) show that although the growth yield of the parental strain when thiosulphate was used as a sulphur source did not reach twice that obtained when methionine was used at the

same concentration, about 1.6-fold more cells were obtained on thiosulphate as compared to methionine. For the mutant strains, similar yields were obtained on the two sulphur sources and, in any case, no increase in the maximal number of cells was observed for growth on thiosulphate as compared to methionine. This is an indication that a wild-type strain uses both sulphur atoms of thiosulphate while *met5*, *met10* and *met20* mutants use only one.

Discussion

The results presented here show that *S. cerevisiae* can use inorganic sulphur sources such as sulphate, sulphite or thiosulphate, as well as organic sulphur sources such as homocysteine, cysteine, methionine and S-adenosyl-methionine. *S. cerevisiae* has also been shown to grow on glutathione (Elskens *et al.*, 1991).

Our evidence also indicates that the only defect of mutants *met1* and *met8* is in sulphite reductase activity. The outcome of this is that the integrity of at least five genes is required for the synthesis of an active sulphite reductase. These genes are *MET1*, *MET5*, *MET8*, *MET10* and *MET20*. The nucleotide sequence of *MET8* has been determined without information being gained as to its function (Cherest *et al.*, 1990).

In *E. coli*, the subunit structure of sulphite reductase has been shown to be $\alpha_8\beta_4$. The α and β subunits are encoded, respectively, by the genes *cysJ* and *cysI* (Ostrowski *et al.*, 1989). In *S. cerevisiae*, the purification and characterization of sulphite reductase has led to the conclusion that the yeast enzyme is also composed of two different subunits (Yoshimoto & Sato, 1968*a, b*, 1970). In

addition, strains no. 6, 11 and 20 from the Naiki collection were found to synthesize a modified inactive sulphite reductase (Yoshimoto & Sato, 1968*b*). Strains no. 6 and 20 have been classified as *met10* mutants (Massetot & de Robichon-Szulmajster, 1975). Moreover, *cysG* mutants of *E. coli* have been shown to lack both nitrite reductase and sulphite reductase activities, due to an impaired synthesis of sirohaem, the prosthetic group of these two enzymes. A specific pathway allows the synthesis of the sirohaem from uroporphyrin III, and at least two enzyme-catalysed steps are needed to transform uroporphyrin III into sirohaem (Warren *et al.* 1990). As yeast does not possess nitrite reductase, these steps are expected to be specific for sulphite reductase in this organism. A mutation in the genes encoding the enzymes catalysing the synthesis of the sirohaem from uroporphyrin III would thus lead to methionine auxotrophic strains. It is thus reasonable to think that among the mutations resulting in the absence of sulphite reductase activity, some could be impairments in sirohaem synthesis.

The genes that encode the enzymes catalysing the different steps of the biosynthesis of sulphur amino acids are shown in Fig. 1. This model links the results presented here and other data recently published by this laboratory. The results obtained with the *met18* and the *met22* mutants still raise some questions. Mutant *met18* accumulates sulphite *in vivo*, showing that sulphite reductase is impaired in this mutant. The same conclusion is reached by assaying sulphite reductase in cell-free extracts. However, *in vivo*, the formation of sulphide from sulphite seems to be partly active in this mutant as assessed by the pale brown colour of this mutant on bismuth medium (see Fig. 2*a*). These observations are in favour of decreased sulphite reductase activity *in vivo* that is not detected *in vitro* due to the low sensitivity of the assay. This low sulphite reductase activity could be due to a modification in the regulation of synthesis of sulphite reductase by the *met18* mutation and is still under investigation.

The reasons for the auxotrophic requirement for organic sulphur of mutant *met22* are not well understood. The enzyme determinations show that a *met22* mutant exhibits wild-type activities of all the enzymes necessary to assimilate sulphate and yet it does not grow on sulphite or on sulphide. Although the *met22* mutant does not grow on sulphite, it is able to concentrate sulphite from YPG-BIG medium as shown by the bismuth test. These observations could reveal differences in the activity or in the regulation of the transport system(s) that allow the transport of sulphate, sulphite and sulphide.

Evidence is presented that all *met* mutants grow on thiosulphate, with the exception of strains mutated in the *MET4* and the *MET25* genes. Thiosulphate metabolism

in *S. cerevisiae* has not yet been studied. In *Salmonella typhimurium*, Nakamura *et al.* (1983, 1984) have shown that mutants defective in cysteine synthase B (encoded by the *cysM* gene) are unable to metabolize thiosulphate, whereas *cysK* mutants devoid of cysteine synthase A can use this metabolite. These authors have shown, moreover, that cysteine synthase B has a sulphocysteine synthase activity *in vitro* that catalyses the formation of *S*-sulphocysteine from *O*-acetylserine and thiosulphate. The pathway for thiosulphate utilization in *Salmonella typhimurium* would thus be: thiosulphate → *S*-sulphocysteine → cysteine (Kredich, 1987). It should be stressed that this pathway is used when thiosulphate is present as a sulphur source and that this sulphur component is not an obligatory intermediate in sulphate assimilation. This is not the case in the genus *Streptomyces*, in which it has been proposed that thiosulphate could be an intermediate in the sulphate assimilation pathway (Kitano *et al.*, 1985; Lydiate *et al.* 1988). In *S. cerevisiae*, the results presented above show that the wild-type strain uses the two sulphur atoms of thiosulphate and that mutants impaired in sulphite reductase activity are capable of using only one sulphur atom of thiosulphate, showing that the utilization of one sulphur atom of thiosulphate involves sulphite reductase. The utilization of the other sulphur atom leads to the formation of sulphide, as assessed by the colour of all *met* mutants when tested on bismuth medium, where formation of sulphide from thiosulphate yields coloured cells. The mechanism of thiosulphate cleavage could involve thiosulphate reductase, which has been purified from yeast (Chauncey & Westley, 1983). Indeed, this enzyme has been shown to cleave thiosulphate yielding sulphite and also sulphide, which can be used by all mutants impaired only in sulphate reduction. This observation explains the inability of the *met25* mutant to use thiosulphate, as it is unable to use sulphite or sulphide. The *MET4* gene encodes the transcriptional activator of all genes implicated in sulphate reduction and sulphur incorporation: a strain bearing a mutation in this gene cannot grow on sulphite or sulphide and consequently cannot use thiosulphate either.

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