Genetic Analysis of a New Mutation Conferring Cysteine Auxotrophy in Saccharomyces cerevisiae: Updating of the Sulfur Metabolism Pathway

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ABSTRACT

We have identified a mutation in a gene of Saccharomyces cerevisiae, STR1, that leads to a strict nutritional requirement for cysteine. The strl-1 mutation decreases to an undetectable level the cystathionine γ-lyase activity. This enzyme catalyzes one of the two reactions involved in the transsulfuration pathway that yields cysteine from homocysteine with the intermediary formation of cystathionine. The phenotype induced by this mutation implies that, in S. cerevisiae, the sulfur atom of sulfide resulting from the reductive assimilation of sulfate is incorporated into a four carbon backbone yielding homocysteine, which, in turn, is the precursor of the biosynthesis of both cysteine and methionine. This also reveals that the direct synthesis of cysteine by incorporation of the sulfur atom into a three carbon backbone as found in Escherichia coli does not occur in S. cerevisiae. The study of the meiotic progeny of diploid strains heterozygous at the STR1 locus has shown that the strl-1 mutation undergoes a particularly high frequency of meiotic gene conversion.

The biosynthesis of the sulfur amino acids, cysteine and methionine, requires first the concentration of sulfate from the medium and its reduction into sulfide. This set of reactions is followed by the incorporation of the sulfur atom of sulfide into a three or a four carbon chain amino acid. Whereas the series of reactions allowing the reduction of sulfate into sulfide appears to be identical in all microorganisms, the assimilation of the sulfur atom in the amino acid backbone is achieved differently, depending on the organism. Indeed, sulfur can be incorporated into a three carbon derivative (a serine ester) yielding cysteine, or in a four carbon derivative (a homoserine ester) yielding homocysteine, or these two mechanisms can exist simultaneously in the same organism.

Homocysteine and cysteine can be converted one into the other by the so-called transsulfuration pathways that transfer the sulfur atom with the intermediary formation of cystathionine. In the C3 to C4 transsulfuration pathway (reactions 3 and 4, Figure 1) cystathionine is synthesized from cysteine and a homoserine ester in a reaction catalyzed by cystathionine γ-synthase. Its cleavage is then catalyzed by cystathionine β-lyase, yielding homocysteine. In the C4 to C3 transsulfuration pathway (reactions 1 and 2, Figure 1), cystathionine is synthesized from homocysteine and serine in a reaction catalyzed by cystathionine β-synthase. It is cleaved by the action of cystathionine γ-lyase, yielding cysteine.

In Escherichia coli, in which the mechanism of sulfur incorporation was clearly established by the study of mutants, sulfide is condensed with a serine ester (O-acetylserine) to yield cysteine (KREDCICH 1987) which is then transformed into homocysteine by the C3 to C4 transsulfuration pathway. The homoserine ester used for the synthesis of cystathionine in this organism is O-succinylhomoserine (COHEN and SAINT-GIRONS 1987).

By contrast, in lower eukaryotes, the reactions allowing the incorporation of sulfur are to date less clearly established. For example, in Aspergillus nidulans, study of mutants has shown that sulfur is incorporated in the C3 backbone yielding cysteine, homocysteine being formed by the C3 to C4 transsulfuration pathway (BALBIN and STEPEN 1974). Nevertheless, direct sulfhydrylation of O-acetylhomoserine by acetylhomoserine sulfhydrylase has also been suggested in A. nidulans (PASZEWSKI and GRABSKI 1974; PIENIAZEK et al. 1974). In Neurospora crassa, another ascomycete, results of regulatory studies favor the incorporation of sulfur at the level of homocysteine (PIOTROWSKA, KRUSZEWSKA and PASZEWSKI 1980).

In Saccharomyces cerevisiae, it is generally admitted that sulfur is incorporated both in O-acetylserine and in O-acetylhomoserine (Figure 1). This arises mainly from the results of YAMAGATA and co-workers, who purified a protein exhibiting both O-acetylhomoserine sulfhydrylase and O-acetylserine sulfhydrylase activities (YAMAGATA, TAKESHIMA and NAIKI 1974, 1975; YAMAGATA 1989). MET25, the structural gene for this O-acetylhomoserine-O-acetylserine sulfhydrylase (reaction 6, Figure 1), has been isolated and studied in our laboratory (SANGSODA, CHEREST and SURDIN-KERJAN 1986; KERJAN, CHEREST and SURDIN-KERJAN 1987; THOMAS, CHEREST and SURDIN-KERJAN 1989). We have reported that a met25 mutant strain is able to grow on homocysteine as well as on cysteine, indi-
cating that the two transsulfuration pathways (C3 to C4 and C4 to C3) are functional in *S. cerevisiae* (Cherest, Eichler and de Robichon-Szulmajster 1969; Masselot and de Robichon-Szulmajster 1975). In addition, only two mutant strains the growth requirement of which can be satisfied only by cysteine have been described up to now. They have been shown to belong to two complementation groups, CYSI and CYS2. First biochemical analysis indicated that *cys1* or *cys2* mutations result in the loss of serine transacetylase activity (HALOS cited in Jones and Fink 1982) and in ONO et al. (1984, 1988). This result was in contradiction with the pathway outlined in Figure 1, because a strain devoid of serine transacetylase activity should be able to synthesize cysteine by the C4 to C3 transsulfuration pathway, unless the *cys1* and *cys2* mutations have a pleiotropic effect impairing this pathway. Recently, ONO and co-workers (1984, 1988) have studied these *cys1* and *cys2* mutants and found that the *cys1* mutant lacked cystathionine γ-lyase activity (catalyzing reaction 2, Figure 1), whereas the *cys2* mutant was devoid of cystathionine β-synthase activity (catalyzing reaction 1, Figure 1). They thus concluded that the *cys1* mutant strain carried a second mutation affecting the gene encoding cystathionine γ-lyase that they called CYS3 and that the *cys2* mutant strain carried a mutation affecting the structural gene of cystathionine β-synthase that they called CYS4. Such an hypothesis explained, in accord with the metabolic pathway presented in Figure 1, that the strains isolated by HALOS require cysteine for growth. But, to account for their phenotypic results, ONO and co-workers (1984, 1988) concluded that CYSI and CYS3 on the one hand and CYS2 and CYS4 on the other hand were tightly linked.

By using a strain bearing a *met25* mutation we anticipated that we could isolate mutant strains impaired in the transsulfuration pathways, by selecting for strains growing exclusively either on cysteine or on homocysteine. A genetic study of these mutants has shown that, in *S. cerevisiae*, cysteine is exclusively synthesized from homocysteine by the C4 to C3 transsulfuration pathway. In addition, this study led to the identification of a genetic locus undergoing meiotic gene conversion with a high frequency.

**MATERIALS AND METHODS**

**Strains:** *S. cerevisiae* strains used in this work are listed in Table 1.

**Media:** YPG medium contained 0.5% yeast extract, 0.5% Bacto-peptone and 3% glucose. YNBG medium contained 7 g/liter of Yeast Nitrogen Base without amino acids and 2% glucose. According to the auxotrophic requirements of strains, uracil (20 μg/ml), histidine (200 μg/ml) and leucine (100 μg/ml) were added to the growth media. B medium was a synthetic medium without any sulfur source. The composition of this medium was the following: (i) mineral salts: 15 mM ammonium chloride, 6.6 mM monopotassium phosphate, 0.5 mM dipotassium phosphate, 1.7 mM sodium chloride, 0.7 mM calcium chloride and 2 mM magnesium chloride; (ii) oligo elements: 0.5 μg/ml boric acid, 0.04 μg/ml copper chloride (1 H₂O), 0.1 μg/ml potassium iodide, 0.19 μg/ml zinc chloride and 0.05 μg/ml ferric chloride (6 H₂O); and (iii) vitamins and growth factors: 2 μg/ml calcium pantothenate, 2 μg/ml thiamine, 2 μg/ml pyridoxine, 0.02 μg/ml biotin and 20 μg/ml inositol. Glucose was added to 2% final concentration. This medium was filter sterilized and a sulfur source was added before use. Inorganic sulfur sources were added at 0.5 mM final concentration. When used as sulfur sources, amino acids were used at the following concentrations: 0.5 mM for L-cysteine, 0.5 mM for dt- homocysteine. To obtain solid B medium, 1% agarose was used instead of agar to minimize the addition of uncontrollable sulfur sources to the medium.

**Genetic methods:** Genetic crosses, sporulation, dissection and the scoring of nutritional markers were as described by Sherman, Fink and Hicks (1987).

**Growth and acellular extracts:** Cells were grown in 10 ml of YNBG medium supplemented to meet the auxotro-

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**Table 1**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>EY9</td>
<td><em>MATa, met25</em></td>
<td>YGSC</td>
</tr>
<tr>
<td>X2180-1A</td>
<td><em>MATa</em></td>
<td>YGSC</td>
</tr>
<tr>
<td>S288C</td>
<td><em>MATa</em></td>
<td>YGSC</td>
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<td>R380</td>
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<td>R. Rothstein</td>
</tr>
<tr>
<td>CC359-OL2</td>
<td><em>MATa, his3, leu2, ura3</em></td>
<td>H. Cherest</td>
</tr>
<tr>
<td>MT1</td>
<td><em>MATa, met25, str-1-1</em></td>
<td>This study</td>
</tr>
<tr>
<td>MT2</td>
<td><em>MATa, met25, str-2-1</em></td>
<td>This study</td>
</tr>
<tr>
<td>CC554-17</td>
<td><em>MATa, str-1</em></td>
<td>This study</td>
</tr>
<tr>
<td>CC554-12</td>
<td><em>MATa, str-1</em></td>
<td>This study</td>
</tr>
<tr>
<td>CC577-2C</td>
<td><em>MATa, str-1-1, leu2</em></td>
<td>This study</td>
</tr>
<tr>
<td>CC555-3B</td>
<td><em>MATa, met25, str-2-1</em></td>
<td>This study</td>
</tr>
<tr>
<td>CC555-25</td>
<td><em>MATa, met25</em></td>
<td>This study</td>
</tr>
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<td>CC591-7</td>
<td><em>MATa, his3, leu2, cys2-1</em></td>
<td>This study</td>
</tr>
<tr>
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<td><em>MATa, cys2-1, CUP1, cys4-1</em></td>
<td>YGSC</td>
</tr>
<tr>
<td>JW4-5C</td>
<td><em>MATa, cys1-3, CUP1, cys3-1</em></td>
<td>YGSC</td>
</tr>
</tbody>
</table>

* Mutation identified by ONO et al. (1984).
* Mutation identified by ONO et al. (1988).

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**FIGURE 1.** Former pathway for the biosynthesis of sulfur amino acids in *S. cerevisiae*. The genes encoding the enzymes catalysing the different reactions are indicated. CYSI, CYS2, CYS3 and CYS4 are indicated as described in HALOS, cited in JONES and FINK (1982) and in ONO et al. (1984, 1988). APS, adenylylsulfate; PAPS, phosphoadenylyl sulfate.
ties of the strains. When the cell concentration reached 10^7 cells/ml, the cultures were centrifuged and washed in potassium phosphate buffer, pH 7.5. The extracts were performed in 100 mM potassium phosphate buffer, pH 7.5, using glass beads as described previously in THOMAS, CHEKEST and SURDIN-KERJAN (1989). The beads and the cell debris were eliminated by centrifugation at 4°C. For the serine transacetylase assay, we tried three different extraction buffers: buffer A contained 100 mM potassium phosphate, pH 7.5, 1 mM phenylmethylsulfonylfluoride (PMSF), and 10% glycerol (v/v). Buffer B contained 100 mM potassium phosphate pH 7.5, 0.1 mM EDTA, and 0.05 mM pyridoxal phosphate (PLP). Buffer C contained 100 mM potassium phosphate, pH 7.5, 0.1 mM PMSF, 0.1 mM EDTA, 0.05 mM PLP and 10% glycerol. To test serine transacylase, acellular extracts were made in an Eaton Press from 250-mL cultures.

Enzymatic assays: O-Acetylhomoserine sulfhydrilase was assayed as described by WIEBERS and GARNER (1967) and the homocysteine formed was estimated according to the method described by KREDICH and TOMKINS (1966). For each extract, four different protein concentrations (2-10 μg) were used. Cystathionine γ-synthase was measured on 250 and 500 μg of protein in a final volume of 250 μl by the method described by KASHIWAMATA and GREENBERG (1970); cystathionine γ-lyase was assayed on 50 and 100 μg of protein in a final volume of 250 μl by the method described in PASZEWSKI and GRABBSKI (1974) and the cysteine synthesized in the reaction was measured as described by GAITONDE (1967). For cystathionine β-lyase assay, we took advantage of the fact that propargylglycine inhibits in vitro cystathionine γ-lyase activity (PIOTROWSKA and PASZEWSKI 1986). Cystathionine β-lyase activity was measured on 50 and 100 μg of protein in a final volume of 100 μl. The reaction mixture contained: 200 mM potassium phosphate buffer, pH 7.5, 12 mM L-cystathionine, 0.5 mM pyridoxal phosphate and 1 mM propargylglycine. The incubation was for 30 min at 37°C and the homocysteine formed was estimated by the method of KREDICH and TOMKINS (1966). Serine transacetylase was assayed by a modification of the methods described by KREDICH and BECKER (1971) and by YAMAGATA (1987). The reaction mixture contained in a total volume of 1 ml, 100 mM potassium phosphate buffer, pH 7.5, 10 mM L-serine, and 0.15 mM acetyl-CoA. For each extract, four different protein concentrations were assayed and a blank without serine was made for each protein concentration. After 30 min of incubation at 30°C, 0.65 mM 5,5′-dithiobis-(2-nitrobenzoic acid) was added. Absorbance was measured at 412 nm. The same assay was run to determine homoserine transacylase activity with homoserine in place of serine. The specific activity was determined, using E (mm) for 1 cm light path = 15.6.

Serine transacetylase has also been assayed by a radioactive assay. The assay mixture was as described above except it was run in 100-μl final volume and serine was radioactive (10 mM final concentration, 2700 cpm/nmol). After incubation, the reaction was stopped by 5 min at 95°C. After centrifugation, 20 μl of the supernatant was layered on a cellulose thin layer chromatography plate. Chromatography was performed in butanol/acetic acid/water (12/30/50 in volume) and radioactive spots were revealed by autoradiography.

Protein concentrations were estimated by the method described by LOWRY et al. (1951).

RESULTS

Isolation of mutants impaired in the transsulfuration pathways: To isolate mutants impaired in the transsulfuration pathways, we mutagenized a strain bearing a met25 mutation. Such a mutation inactivates the synthesis of the sulfur amino acids from the sulfur arising from the reduction of sulfate.

The strain EY9 was submitted to UV mutagenesis to obtain a 10% survival. Then 15,000 colonies were tested for their ability to grow on cysteine or homocysteine. One strain was found to grow exclusively on cysteine (strain MT1) and one was shown to grow exclusively on homocysteine (strain MT2).

Genetic and phenotypic analysis of the mutant strain MT2: The mutant MT2 which grows on homocysteine but not on cysteine was crossed to the wild type strain X2180-1A. The diploid strain (CC555) was sporulated and 15 tetrads were analyzed. The ability to grow on cysteine segregated as a two gene trait (Table 2). These results show that probably the isolated mutation results in the inability to grow on cysteine only in the presence of a met25 mutation. A segregant of cross CC555, exhibiting the same phenotype as strain MT2 was thus backcrossed to a met25 mutant strain. The resulting diploid (CC579) was sporulated and 16 tetrads analyzed. Results (Table 2) show that, in this background, the inability to grow on cysteine segregated perfectly in a 2+/2- pattern, proving that the inability to grow on cysteine segregates as a monogenic trait. The gene, the mutation of which leads to a strict homocysteine requirement in the presence of a met25 mutation, has been called STR2 (for Sulfur TRansfer in accord with R. K. MORTIMER).

Phenotypic analysis of a strain bearing the str2-1 mutation has been made on YNBG and B media (Table 3). It must be recalled that in the B medium, the added amino acid is the only sulfur source. The results show that such a strain is able to use homocysteine as a sulfur source (B medium + homocysteine). By contrast, a str2-1 mutant cannot use cysteine as a sulfur source (B medium + cysteine) proving that the C3 to C4 transsulfuration pathway (cysteine to homocysteine) is impaired in this mutant. Moreover, the str2 mutant grows on YNBG medium without any addition, as this medium contains sulfate that can be

| TABLE 2 |

<table>
<thead>
<tr>
<th>Diploid</th>
<th>Addition to minimal YNBG medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>CC555</td>
<td>15</td>
</tr>
<tr>
<td>CC579</td>
<td>16</td>
</tr>
</tbody>
</table>

The concentration of bio-homocysteine was 0.2 nM and of L-cysteine was 0.5 mM. CC555 = MT2 × X2180-1A (met25,str2/STR2); CC579 = CC555-3B × CC555-2C (met25,str2/str2).
required cysteine and complemented the allows growth on cysteine and not on homocysteine. Seventeen spores grew only on cysteine supplemented medium, which is the phenotype of the strain MT1: The mutant MT1 which grows on cysteine but not on homocysteine was crossed to the wild type strain X2180-1A. The diploid (CC554) was sporulated but no complete tetrad germinated. Among 31 random spores, fourteen could grow on minimal medium supplemented with homocysteine or with cysteine, which is the phenotype of the strain MT1 bearing both a met25 mutation and a mutation that allows growth on cysteine and not on homocysteine. However, some of these 17 strains could carry only the newly isolated mutation if this mutation resulted by itself in a cysteine requirement. To determine if the strains that grow only on cysteine carried the met25 mutation, complementation tests were made. Diploids were constructed between the 17 strains growing exclusively on cysteine and a met25 mutant. Among the 13 strains that gave diploids with the met25 mutant, ten did not complement with the met25 mutant whereas three did. Two of the three strains that required cysteine and complemented the met25 mutation were crossed to wild-type strains, yielding the diploid strains CC575 and CC577, which were sporulated. Phenotypic analyses of 48 tetrads from diploid CC575 and 61 tetrads from diploid CC577 are shown in Table 4. In the case of diploid CC575, the His-, Ura+ and Leu- characters showed a perfect 2+/2- segregation. Likewise, the Leu+ and His- characters involved in cross CC577 segregated perfectly 2+/2-. As expected, all segregants from CC555 and from CC577 grow on a cysteine supplemented medium. For the cysteine requirement, 43 tetrads out of 48 in cross CC575 and 50 tetrads out of 61 in cross CC577 exhibit a 2+/2- segregation. Nevertheless, in both crosses an equal number of 3+/1- and of 1+/3- tetrads were found. This segregation pattern is typical of meiotic gene conversion and is not compatible with the segregation of two linked genes. All spores from diploids CC575 and CC577 were tested on the sulfurless B medium supplemented with homocysteine. Only strains able to synthesize cysteine from homocysteine, thus having an active C4 to C3 transulfuration pathway, can grow on such a medium. The segregation on this medium was the same as in the YNB minimal medium showing that, in the tetrads exhibiting 3+/1- and 1+/3- segregation of the cysteine marker, growth corresponds in all cases to a functional C4 to C3 transulfuration pathway and the absence of growth results from an impaired C4 to C3 transulfuration pathway.

In conclusion, the analysis of the progeny of diploids CC575 and CC577 proves that the cysteine requirement results from a mutation affecting a single gene. This gene has been named STR1. The ability of strains bearing the str1-1 mutation to grow on media differently supplemented is summarized in Table 3.

Enzymatic analysis: O-Acetylhomoserine sulfhydrylase, cystathionine-β-synthase, cystathionine γ-lyase, and cystathionine β-lyase have been assayed in different mutant strains. The main result of this enzymatic study (Table 5) is that the str1-1 mutants are devoid of cystathionine γ-lyase activity. All strains exhibit comparable activities for the other enzymes with the exception of strains bearing a mutation in gene MET25, which are devoid of acetylhomoserine sulfhydrylase activity, MET25 being the structural gene for this enzyme (KERJAN, CHEREST and SURDIN-KERJAN 1987). These results show also that the strain CC555-1D, bearing a mutation in the STR2 gene, has a wild-type cystathionine β-lyase activity. According to the results reported above, this strain could be impaired in the C3 to C4 transulfuration pathway, which is comprised of two steps catalyzed respectively by cystathionine γ-synthase and by cystathionine β-lyase. As the str2-1 mutant exhibits cystathionine β-lyase activity, this mutation affects probably the gene encoding cystathionine γ-synthase. This point could not be enzymatically verified because no assay conditions have been found for this enzyme in yeast.

Genetic and phenotypic analysis of strain JW4-5C: The results reported here show that a strain bearing the str1-1 mutation has no cystathionine γ-

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**TABLE 3**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Addition to YNBG minimal medium</th>
<th>Addition to B medium</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>Hcy</td>
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<tr>
<td>X2180-1A</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CC559-OL2</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R280</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>EY9</td>
<td>met25</td>
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<td>-</td>
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<td>CC554-17</td>
<td>str1-1</td>
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<td>+</td>
</tr>
<tr>
<td>CC554-12</td>
<td>str1-1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
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<td>str2-1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>str1-1,met25</td>
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<td>+</td>
</tr>
<tr>
<td>MT2</td>
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<td>+</td>
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</table>

Growth was noted after 48 hr at 30°C. + indicates good growth, - indicates no growth. Hcy, DL-homocysteine 0.2 mM; Cyst, L-cysteine 0.5 mM.

**TABLE 4**

<table>
<thead>
<tr>
<th>Diploid</th>
<th>No. of tetrads tested</th>
<th>Types of tetrads, tested on minimal YNBG medium</th>
</tr>
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<tbody>
<tr>
<td>CC575</td>
<td>48</td>
<td>2+/2-</td>
</tr>
<tr>
<td>CC577</td>
<td>61</td>
<td>2+/2- 3+/1- 1+/3- 43 50 4 3 6 5</td>
</tr>
</tbody>
</table>

CC575 = CC559-OL2 × CC554-12 (STR1/str1); CC577 = R280 × CC554-17 (STR1/str1).
The activities are expressed as nmoles of substrate transformed.

**TABLE 5**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Addition to MM</th>
<th>CTT (\beta)-synth</th>
<th>CTT (\gamma)-lyase</th>
<th>OAH Sulff</th>
<th>CTT (\beta)-lyase</th>
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<tbody>
<tr>
<td>CC539-OL2</td>
<td></td>
<td>l-cys</td>
<td>18.5</td>
<td>5.9</td>
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<td>(str1)</td>
<td>l-cys</td>
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<td>168</td>
<td>—</td>
</tr>
<tr>
<td>CC577-5B</td>
<td>(str1)</td>
<td>l-cys</td>
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<td>0.6</td>
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<td>—</td>
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<tr>
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<td>0.3</td>
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<tr>
<td>CC555-1B</td>
<td>(met25)</td>
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<tr>
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<td>(cys2)</td>
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<td>l-cys</td>
<td>12</td>
<td>0.6</td>
<td>241</td>
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</table>

MM, YNBG-based minimal medium l-cys: 0.2 mM l-cysteine; DL-Hcys, 1 mM DL-homocysteine; CTT \(\beta\)-synth, cystathionine \(\beta\)-synthase; CTT \(\gamma\)-lyase, cystathionine \(\gamma\)-lyase; CTT \(\beta\)-lyase, cystathionine \(\beta\)-lyase; OAH Sulff, O-acetyl homoarginine sulfhydraslyase. The activities are expressed as nmoles of substrate transformed/min/mg protein\(^{-1}\). — indicates not determined.

Cystathionine \(\gamma\)-lyase activity in different diploid strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Cystathionine (\gamma)-lyase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC630</td>
<td>(STR1,CYS3)</td>
<td>6.8</td>
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<tr>
<td>CC619</td>
<td>(str1,CYS4/STR1,cys4)</td>
<td>3.6</td>
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<tr>
<td>CC620</td>
<td>(str1,CYS3/STR1,cys3)</td>
<td>0.9</td>
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</table>

Segregation of the cysteine requirement in the progeny of diploid CC620 is not compatible with the segregation of two independent genes. Such a segregation could nevertheless result either from the segregation of two linked genes or from meiotic gene conversion events between two alleles of the same gene as we have shown that both the \(str1\) allele and the \(cys3\) mutation present in strain JW4-5C exhibit a high frequency of gene conversion. Moreover, all spores derived from diploid CC620 were tested on B medium supplemented with homocysteine. The segregation was the same as on the surface containing medium (YNBG medium, see MATERIALS AND METHODS), showing that the cysteine requirement results in all cases from an impaired C4 to C3 transsulfuration pathway.

By genetic analysis we could not formally show the identity of the \(STR1\) and \(CYS3\) genes. We thus assayed cystathionine \(\gamma\)-lyase activity in an extract of diploid CC620. Indeed intragenic functional complementation between two different mutated alleles of the same gene results frequently in an inactive enzyme in vitro. Results reported in Table 6 show that diploid CC620 has a very low cystathionine \(\gamma\)-lyase activity, which is in favor of \(STR1\) and \(CYS3\) being the same gene. This table shows also a perfect gene dosage of this enzymatic activity in the \(STR1/STR1\) and in the \(STR1/str1\) diploids, which is a good indication that \(STR1\) is the structural gene for cystathionine \(\gamma\)-lyase. Another implication of these experiments is that cystathionine \(\gamma\)-lyase encoded by \(STR1\) that displays intragenic complementation, is likely to be at least a dimer.

Strain JW4-5C bears a single mutation that results in (1) a strict cysteine auxotrophy, (2) a lack of cystathionine \(\gamma\)-lyase activity and (3) a high frequency of meiotic gene conversion. Moreover, diploid CC620 (\(str1/cys3\), although prototrophic, exhibits a very low cystathionine \(\gamma\)-lyase activity in vitro. It thus appears that strain JW4-5C bears a mutation affecting the \(STR1\) gene, which we will indicate as \(str1\).2.

**Genetic analysis of strain JW1-1C:** Strain JW1-1C has been shown by ONO et al. (1988) to be devoid of serine transacetylase activity and of cystathionine \(\beta\)-synthase, which catalyses the first reaction of the C4 to C3 transsulfuration pathway. One prediction from the results we report in the preceding sections is that a single mutation in the gene encoding cystathionine \(\beta\)-synthase should result in a strict requirement for cysteine. We performed a genetic analysis of strain
JW1-1C. It was crossed to strain R280, a diploid (CC591) was sporulated and its meiotic progeny was analysed. No complete tetrads were obtained. Nevertheless one spore (CC591-7) exhibiting the same phenotype as JW1-1C was crossed to the wild type strain S288C. One diploid (CC604) was sporulated and 72 tetrads were analyzed. They all segregated perfectly 2+2- for the histidine and the leucine requirements. For the cysteine requirement 69 tetrads segregated 2+/2- 2 tetrads were of the 3+/1- type and one was of the 1+/3- type. This segregation pattern is typical of meiotic gene conversion events occurring at a low frequency but not of the segregation of two genes. Moreover, the growth pattern of all spores was the same on B medium containing homocysteine as on the YNBG medium, proving that prototrophy is due in all cases to an active C4 to C3 transsulfuration pathway. On the contrary, our results show that a unique impairment in the C4 to C3 transsulfuration pathway always results in a cysteine requirement.

**The metabolism of sulfur amino acids in S. cerevisiae:**

The results reported above show that in a strain of *S. cerevisiae*, a single mutation impairing the C4 to C3 transsulfuration pathway results in a strict growth requirement for cysteine. The consequence of this finding is that in *S. cerevisiae*, the C4 to C3 transsulfuration pathway is the only passage to cysteine biosynthesis. It follows that the pathway for sulfur amino acids biosynthesis in *S. cerevisiae* should be represented as in Figure 2, with the incorporation of the sulfur atom only in the four carbon backbone. One prediction of this model is that serine transacetylase plays no role in the biosynthesis of cysteine. We decided to investigate this hypothesis.

**Serine transacetylase:** We have assayed serine transacetylase in different strains. The striking result of these enzymatic determinations is that we were unable to find any serine transacetylase activity whatever the strain may be. Even in the wild type strains serine transacetylase was undetectable. To verify these results, we performed different experiments. In the first one, cells from strain CC359-OL2 (wild type for the sulfur amino acids metabolism) were extracted in the three different buffers described under MATERIALS AND METHODS. In the second one, we grew the wild-type strains used in the present work (X2180-1A, CC359-OL2 and R280) and made extracts in the stabilizing buffer of ONO et al. (1988) (buffer B described in MATERIALS AND METHODS). In another attempt to find serine transacetylase activity, we devised a new assay using radioactive serine as described under MATERIALS AND METHODS. But in all cases no serine transacetylase activity could be detected. For each acellular extract, homoserine transacetylase activity was assayed as a control by adding homoserine in place of serine in the assay and in each case an homoserine transacetylase activity was measured. The absence of detectable serine transacetylase activity in wild-type strains is in accord with the pathway shown in Figure 2.

TABLE 7

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth on B medium + homocysteine</th>
<th>Cystathionine ß-synthase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC604-18A</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>CC604-18B</td>
<td>+</td>
<td>30</td>
</tr>
<tr>
<td>CC604-18C</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CC604-18D</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>

The relevant genotype of diploid CC604 (CC591-7 × S288C) is: cys4/CYS4.

FIGURE 2.—Metabolism of sulfur amino acids in *S. cerevisiae*. For abbreviations see legend of Figure 1.
in Figure 2 based on our finding that cysteine auxotrophy is the result of the impairment of the C4 to C3 transsulfuration pathway.

The absence of serine transacetylase activity in vitro cannot be taken as a formal proof of its absence in vivo. However, our physiological argument against its existence is that the strl-1 mutant fails to grow on sulfate. But one possible flaw of this experiment is that the strl-1 mutation could block sulfate uptake or reduction. To show that sulfate uptake and reduction was active in the strl-1 mutant, we crossed it to the str2-1 mutant. The resulting diploid (CC587) was sporulated and 12 tetrads analyzed. The spores that needed both homocysteine and cysteine for growth on B medium, bore simultaneously the strl-1 and str2-1 mutations. In all cases, these double mutants could grow on YNBG medium supplemented with cysteine, showing that they could use the sulfate contained in the YNBG medium to synthesize homocysteine.

**DISCUSSION**

Genetic, phenotypic and enzymatic analyses of a new mutant of *S. cerevisiae* that exhibits a strict nutritional requirement for cysteine and that is impaired in a gene encoding cystathionine γ-lyase that we called STR1 are reported. The use of a sulfur-controlled medium has confirmed that this cysteine requirement is due to the inactivation of the C4 to C3 transsulfuration pathway. Such a result shows that sulfur is incorporated in a C4 backbone and that cysteine is exclusively synthesized from homocysteine in *S. cerevisiae*. The pathway to sulfur amino acids biosynthesis should thus be represented as in Figure 2.

This report also reveals that the str2-1 mutation impairing the C3 to C4 transsulfuration pathway does not result in any growth requirement in a minimal medium containing sulfate. This result is also in accord with the pathway outlined in Figure 2. Indeed, as cysteine is not an obligatory intermediary metabolite in the biosynthesis of methionine in *S. cerevisiae*, impairment of the C3 to C4 transsulfuration pathway does not induce a visible phenotype in a wild type background.

Supporting our model of sulfur amino acids metabolism is the action of propargylglycine, an antibiotic that has a lethal effect on *S. cerevisiae* (PIOTROWSKA and PASZEWSKI 1986). Indeed, it has been shown to inactivate specifically cystathionine γ-lyase in rats (BEATTY and REED 1980), although other enzymes can be affected to a lesser extent (BURNETT, MACCOTTE and WALSH 1980; TANASE and MORINO 1976). In *S. cerevisiae*, propargylglycine inhibits the activity of yeast cystathionine γ-lyase in vitro (PIOTROWSKA and PASZEWSKI 1986). We have performed experiments showing that it inhibits growth of the wild-type strain X2180-1A and that this effect can be reversed by the addition of cysteine to the medium but not by the addition of methionine (results not shown). These data can be explained by the fact that cysteine is only synthesized by the C4 to C3 transsulfuration pathway.

We also report here that serine transacetylase activity could not be detected in wild type strains. Although not a formal proof of the absence of this enzyme in *S. cerevisiae*, this result is in accord with the cysteine requirement of strains impaired only in the C4 to C3 transsulfuration pathway. We thus propose that serine transacetylase is normally not synthesized in *S. cerevisiae*. Moreover, a strain mutated only in the STR1 gene does not grow on a medium supplemented with O-acetylserine and sulfate, indicating that O-acetylhomoserine sulfhydrylase does not function in vivo as an O-acetylserine sulfhydrylase (results not shown).

The model we propose for sulfur amino acid metabolism in *S. cerevisiae* involves incorporation of sulfur only in homocysteine by a reaction catalysed by the MET25 encoded enzyme. It follows that the MET25 encoded enzyme does not catalyse the incorporation of sulfur in a three carbon backbone, as postulated up to now. On the contrary, in *E. coli* the incorporation of sulfur is in O-acetyl serine yielding cysteine. In *E. coli*, two enzymes encoded respectively by cysK and cysM can catalyse the sulfhydrylation of O-acetylserine to cysteine. Comparison of the polypeptide sequences deduced from the gene MET25 of *S. cerevisiae* (KERJAN, CHEREST and SURDIN-KERJAN 1987) and from genes cysK and cysM of *E. coli* (BYRNE et al. 1988; SIRKO et al. 1990) has revealed no similarities between the yeast enzyme and the *E. coli* enzymes, whereas similarities have been found between the MET25 protein and two enzymes catalysing the C3 to C4 transsulfuration pathway in *E. coli* (BELFAIZA et al. 1986). This is in accord with our finding that the MET25 protein (acetylhomoserine sulfhydrylase) does not catalyse in vivo the sulfhydrylation of O-acetylserine.

The two transsulfuration pathways are functional in *S. cerevisiae*. Nevertheless, we can ask if they can be concomitantly active. This system in which the forward and reverse reactions are catalysed by different enzymes is not unique but it seems that its study will bring elements to the knowledge of the evolution of pathways. The regulation of the transsulfuration pathways are presently under study in our laboratory.

In the study of the meiotic progeny of heterozygous diploid strains at the STR1 locus, we have obtained a few tetrads showing irregular segregation for the cysteine requirement. We observed only 3+1/1+3 aberrant tetrad classes, which is typical of gene conversion. (FOGEL, MORTIMER and LUSNAK 1981). The strl-1 allele that we have described here displays a high conversion frequency (about 15%). Such a frequency of gene conversion is particularly high. The gene conversion frequency for the strl-2 allele (in
strains JW4-5C is about 5%, an expression of the well
known polarity of conversion (Fogel, Mortimer and
Lusnak 1981). Gene STR1 could thus be a good
substrate for a study of gene conversion at the mole-
cular level.

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