JOURNAL OF BACTERIOLOGY, Sept. 1993, p. 5366–5374
0021-9193/93/175366-09$02.00/0

Vol. 175, No. 17

Cysteine Biosynthesis in *Saccharomyces cerevisiae* Occurs through the Transsulfuration Pathway Which Has Been Built Up by Enzyme Recruitment

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Received 14 April 1993/Accepted 15 June 1993

The transsulfuration pathways allow the interconversion of homocysteine and cysteine with the intermediary formation of cystathionine. The various organisms studied up to now incorporate reduced sulfur into a three- or a four-carbon chain and use differently the transsulfuration pathways to synthesize sulfur amino acids. In enteric bacteria, the synthesis of cysteine is the first step of organic sulfur metabolism and homocysteine is derived from cysteine. Fungi are capable of incorporating reduced sulfur into a four-carbon chain, and they possess two operating transsulfuration pathways. By contrast, synthesis of cysteine from homocysteine is the only existing transsulfuration pathway in mammals. In *Saccharomyces cerevisiae*, genetic, phenotypic, and enzymatic study of mutants has allowed us to demonstrate that homocysteine is the first sulfur amino acid to be synthesized and cysteine is derived only from homocysteine (H. Cherest and Y. Surdin-Kerjan, Genetics 130:51–58, 1992). We report here the cloning of genes *STR4* and *STR1*, encoding cystathionine β-synthase and cystathionine γ-lyase, respectively. The only phenotypic consequence of the inactivation of *STR1* or *STR4* is cysteine auxotrophy. The sequencing of gene *STR4* has allowed us to compare all of the known sequences of transsulfuration enzymes and enzymes catalyzing the incorporation of reduced sulfur in carbon chains. These comparisons reveal a partition into two families based on sequence motifs. This partition mainly correlates with similarities in the catalytic mechanisms of these enzymes.

The increasing knowledge of related biosynthetic pathways common to numerous organisms emphasizes the prevalent occurrence of metabolic diversity. Molecular studies of metabolic diversity now offer a propitious basis for insight into the evolutionary mechanisms that allowed acquisition of multistep pathways. Two major working hypotheses have been proposed to describe the establishment of such pathways. The first one (12) invokes a retrieval recruitment of new enzymes progressively built backwards from the final metabolite of the pathway. The second one (15, 38) suggests that primitive enzymes possessed a very broad specificity permitting subsequent elaboration of new enzymes following gene duplication. The latter hypothesis has received some support from the observed sequence similarities between enzymes catalyzing consecutive steps in the isoleucine biosynthetic pathway (25).

Transsulfuration metabolism is a well-documented case of a pathway exhibiting alternative means whereby various organisms synthesize their metabolites. The transsulfuration pathways allow the interconversion of homocysteine and cysteine with the intermediary formation of cystathionine (Fig. 1). Enteric bacteria derive the sulfur moiety of homocysteine solely from cysteine. Fungi are capable of incorporating reduced sulfur into a four-carbon chain, and they possess two operating transsulfuration pathways. By contrast, synthesis of cysteine from homocysteine is the only existing transsulfuration pathway in mammals (Fig. 1). Moreover, alternative carbon substrates are used for these syntheses, depending on the organism: the four-carbon ester involved in homocysteine synthesis could be either *O*-succinylhomoserine, *O*-acetylhomoserine, or phosphohomoserine (for a review, see reference 37). Likewise, the three-carbon chain utilized for cysteine synthesis can be esterified or not (36).

In addition to the biochemical diversity of transsulfuration metabolism among extant species, it is well known that in vitro, transsulfuration enzymes exhibit broad specificities. For example, *Escherichia coli* cysteine synthase and yeast homocysteine synthase are both capable of catalyzing various reactions: the former can use triazole as a substrate (18), while the latter can incorporate reduced sulfur into a three-carbon chain (37). Cystathionine γ-synthase and cystathionine β-lyase both can use cystine as a substrate. Deamination of L-serine may also be carried out by cystathionine β-lyase (3). It must be noted that all of the transsulfuration enzymes utilize pyridoxal phosphate as a common cofactor.

Fungi appear to be the organisms which retain the most complex transsulfuration metabolism (Fig. 1). Genetic, phenotypic, and enzymatic study of *Saccharomyces cerevisiae* mutants allowed us to demonstrate that in yeast cells, cysteine derives only from homocysteine (5). Acquisition of molecular data on the yeast transsulfuration metabolism thus seems propitious for defining evolutionary relationships between related proteins that no longer serve the same function.

We report here the cloning and sequencing of gene *STR4* as well as the study of gene *STR1*, encoding cystathionine β-synthase and cystathionine γ-lyase, respectively. Phenotypic consequences of the inactivation of each gene are described. Comparisons of all known sequences of transsulfuration enzymes reveals a partition into two families based on sequence motifs. This partition mainly correlates with similarities in the catalytic mechanisms of these enzymes.

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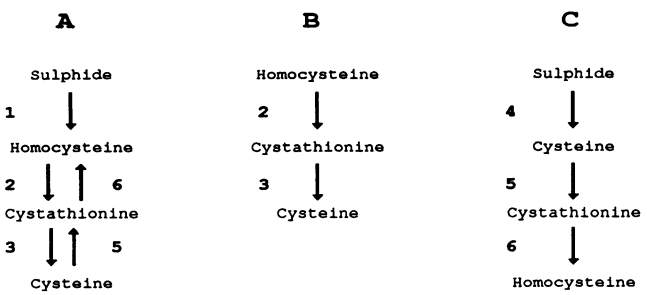

FIG. 1. Existing transsulfuration pathways in *S. cerevisiae* (A), mammals (B), and *E. coli* (C). Enzymes: 1, homocysteine synthase; 2, cystathionine β-synthase; 3, cystathionine γ-lyase; 4, cysteine synthase; 5, cystathionine γ-synthase; 6, cystathionine β-lyase.

MATERIALS AND METHODS

Strains, media, and microbiological techniques. *E. coli* HB101 and JM103 were used as hosts for plasmid maintenance. *S. cerevisiae* strains used in this work are listed in Table 1. To grow *E. coli*, we used media described by Maniatis et al. (21). For *S. cerevisiae*, YPG and YNBG media were as described previously (5). Glutathione was used in some experiments as a source of cysteine (8). According to the auxotrophic requirements of strains, uracil (20 μg/ml), adenine (40 μg/ml), histidine (200 μg/ml), leucine (100 μg/ml), and tryptophan (20 μg/ml) were added to the growth media. *E. coli* was transformed as described by Cohen et al. (7). *S. cerevisiae* was transformed after lithium chloride treatment as described by Ito et al. (14). Genetic crosses, sporulation, dissection, and scoring of nutritional markers were done as described by Sherman et al. (30).

Plasmid vectors. The multicopy plasmid pEMBLYe23 and the integrative plasmid pEMBLYi22, both bearing gene *URA3* as a selectable marker, were used (1). The *S. cerevisiae* genomic library used for the cloning of genes *STR*1 and *STR*4 was previously described (35).

Plasmid integration and genetic analysis. To show that the insert present in plasmid pSTR4-2 was able to direct plasmid integration to a site on the genome linked to gene *STR*4, we constructed the following plasmid. The sequences from the 2μm plasmid were deleted from plasmid pSTR4-2, yielding plasmid pSTR4-01. Plasmid pSTR4-01 was linearized by *Xba*I to direct the integration to the homologous genomic sequences and used to transform strain W303-1A (*ura3 STR*4). One resulting Ura^+ transformant was crossed to strain CC645-23C (*ura3 str4-1*). The diploid was sporulated, and its meiotic progeny was analyzed in 20 tetrads. In 19 tetrads, all auxotrophic characters segregated perfectly $2^+/2^-$, and glutathione-positive (Glt^+) spores were all Ura^+, showing genetic linkage between the mutant locus and the integrated cloned DNA. One tetrad segregated $3^+/1^-$ for the glutathione auxotrophy, and one of the Glt^+ spores was Ura^-. This can be accounted for by the high frequency of gene conversion evidenced at the *STR*4 locus (5).

To show that the insert present in plasmid pSTR1-1 was able to direct plasmid integration to a site on the genome linked to gene *STR*1, we constructed the following plasmid. The *Hin*dIII-*Xho*I fragment of plasmid pSTR1-1 was inserted in the *Hin*dIII and *Sal*I sites of plasmid pEMBLYi22. The resulting plasmid, pSTR1-01, was linearized by *Sal*I and was used to transform strain W303-1A (*ura3 STR1*) to uracil prototrophy. One resulting Ura^+ transformant was crossed to strain CC659-4B (*ura3 str1-1*). The diploid was sporulated, and its meiotic progeny was analyzed in 18 tetrads. In 16 tetrads, a perfect $2^+/2^-$ segregation was observed for all characters, and all Glt^+ spores were Ura^+, showing genetic linkage between the mutant locus and the cloned DNA. Two tetrads exhibited a $3^+/1^-$ segregation for the glutathione auxotrophy, and in each tetrad one Glt^+ spore was Ura^-. Again, this results from the high frequency of meiotic gene conversion reported for the *STR1* locus (5).

Recombinant DNA methods. Plasmid purification was performed as described by Ish-Horowicz and Burke (13). Genomic DNA analysis was performed with DNA prepared as described by Hoffman and Winston (11). Probes were made radioactive by the random-priming method described by Hodgson and Fisk (10).

Systematic deletion subclones were generated by the method described by Thomas and Surdin-Kerjan (34). Single-stranded phage DNA prepared from these deletions was sequenced by using the Pharmacia T7 sequencing kit. Analysis of the DNA sequence and comparisons on a VAX computer were made possible by the computer facilities of CITI2 in Paris.

Enzymatic assays. Cystathionine β-synthase and cystathionine γ-lyase were assayed as described previously (5). Protein concentrations were estimated by the method described by Lowry et al. (20).

Nucleotide sequence accession number. The nucleotide sequence of gene *STR4* reported here has been assigned EMBL accession number X72922.

TABLE 1. Strains used

Strain	Genotype	Source
W303-1A	*MAT***a** *ade2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein
W303-1B	*MATα ade2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein
CC627-2B	*MATα his3 ura3 str4-1*	This work
CC645-23C	*MAT***a** *trp1-1 ura3 str4-1*	This work
CC639-7D	*MAT***a** *leu2 ura3 str1-1*	This work
CC659-4B	*MATα his3 ura3 str1-1*	This work
C180	*MAT***a** *ade2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 str4::URA3*	This work
C181	*MAT***a** *ade2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 str1::URA3*	This work

RESULTS

Cloning and sequencing of the cystathionine β-synthase-encoding gene from *S. cerevisiae*. The cystathionine β-synthase gene (*STR4*) from *S. cerevisiae* was cloned by complementation of the glutathione auxotrophy of strain CC627-2B. The strain was transformed with the gene library described in Materials and Methods. Among 18,000 Ura^+ transformants tested, three strains were found to grow without glutathione. Two clones harbored identical plasmids with a 7-kbp insert. The third one harbored a plasmid with a 8.7-kbp insert partly overlapping the first insert. The 7-kbp insert was subcloned, and the sequences required to complement the *str4* mutation of strain CC627-2B were mapped to the *Hin*dIII-*Sph*I fragment (Fig. 2). To ascertain that gene *STR4* was cloned, we verified that the DNA sequences of the insert of plasmid pSTR4-2 were able to direct the integration of the plasmid to a site linked to gene *STR4* on the genome (see Materials and Methods).

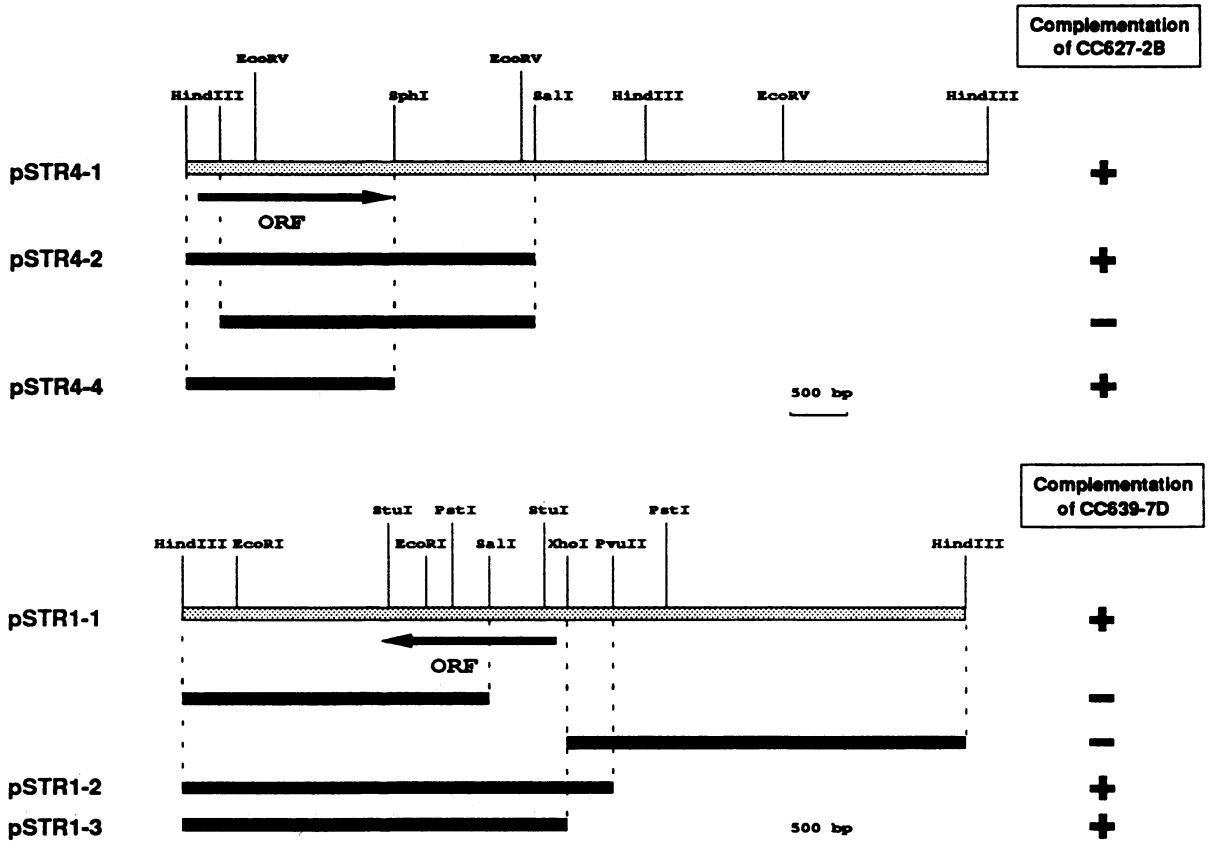

FIG. 2. Physical maps of the *STR1* and *STR4* regions. Fragments subcloned in plasmid pEMBLYe23 as well as their abilities to complement the *str1* mutation (strain CC639-7D) or the *str4* mutation (strain CC627-2B) are shown. ORF, open reading frame.

Cystathionine β-synthase was assayed in strain CC627-2B (*str4*) transformed with plasmid pSTR4-1. In extracts of such a strain, a 10-fold increase of cystathionine β-synthase activity over that of the parental wild-type strain was measured, in agreement with cystathionine β-synthase structural gene being expressed from a multicopy plasmid (Table 2).

The *Hin*dIII-*Sph*I fragment of plasmid pSTR4-2 was sequenced as described in Materials and Methods. The sequence was entirely determined on both strands. The nucleotide sequence and the polypeptide sequence deduced from the longest open reading frame are shown in Fig. 3.

Cloning of the cystathionine γ-lyase-encoding gene from *S. cerevisiae*. The cystathionine γ-lyase gene (*STR1*) was cloned by complementation of the glutathione auxotrophy of strain CC639-7D. The strain was transformed with the gene library described in Materials and Methods. Among 16,000 Ura$^+$ transformants tested, six strains were found to grow without glutathione. These six strains harbored the same plasmid with a 6.6-kbp insert. By subcloning, the sequences able to complement the glutathione auxotrophy of strain CC639-7D were mapped to the 3.4-kbp *Hin*dIII-*Xho*I fragment (Fig. 2). To ascertain that gene *STR1* was cloned, we verified that the DNA sequences of the insert of plasmid pSTR1-1 were able to direct the integration of the plasmid to a site linked to gene *STR1* on the genome (see Materials and Methods).

Cystathionine γ-lyase was assayed in strain CC639-7D (*str1*) transformed with plasmid pSTR1-1. In extracts of such a strain, a 17-fold increase of cystathionine γ-lyase activity over that of the parental wild-type strain was measured, in agreement with cystathionine γ-lyase structural gene being expressed from a multicopy plasmid (Table 2).

While this work was being carried out, Ono et al. (24) reported the cloning and sequencing of the *CYS3* gene from *S. cerevisiae*. The restriction map of this gene is identical with that of *STR1* reported here. This finding confirms that the two loci represent the same gene, the conclusion previously reached by our genetic analysis (5).

Gene disruption alleles at either *STR1* or *STR4* result in cysteine auxotroph yeast strains. Gene disruption alleles at *STR1* and *STR4* were constructed as described by Rothstein (27). To disrupt gene *STR1*, the 1.1-kbp *Stu*I fragment of plasmid pSTR1-01 (described in Materials and Methods) was replaced by the *URA3* gene. The resulting plasmid was cut by *Hin*dIII and used to transform strain W303-1A to uracil

TABLE 2. Enzyme activities of different strains

Strain	Relevant genotype	Plasmid present	Enzyme activity (nmol of substrate transformed/min/mg of protein)	
			Cystathionine β-synthase	Cystathionine γ-lyase
W303-1B			30	9.6
CC627-2B	*str4*		0	7.2
CC627-2B	*str4*	pSTR4-1	377	8.8
CC639-7D	*str1*		38	<1
CC639-7D	*str1*	pSTR1-1	42	178

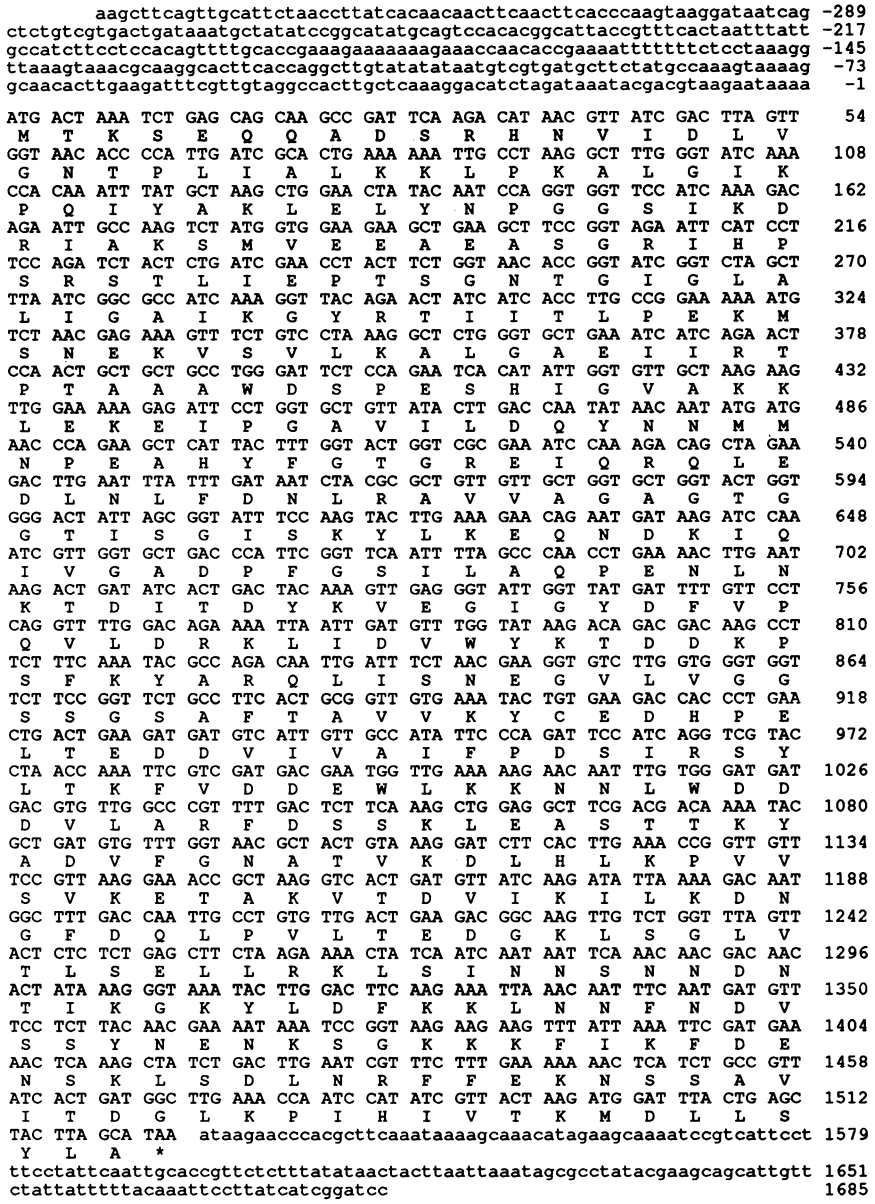

FIG. 3. Nucleotide and deduced amino acid sequences of the *STR4* gene with the 5′ and 3′ flanking regions. The noncoding strand is shown. The nucleotide sequence is numbered from nucleotide 1 of the presumed initiation codon.

prototrophy. DNA blot analyses of genomic DNA isolated from the parental strain W303-1A and from one of the transformed strains (C181) are shown in Fig. 4a. In strain C181, a 1.2-kbp fragment is detected as expected from the cleavage at the *Eco*RV site in the *URA3* sequence instead of the 3.9-kbp fragment revealed in the parental strain W303-1A. To disrupt gene *STR4*, the *Xba*I-*Eco*RI fragment of pSTR4-01 (described in Materials and Methods) was replaced by the *URA3* gene. The resulting plasmid was cut by *Hin*dIII and used to transform strain W303-1A to uracil prototrophy. DNA blot analyses of genomic DNA isolated from the parental strain W303-1A and from one of the transformed strains (C180) are shown in Fig. 4b. In the case of strain W303-1A, one band accounting for the two expected *Eco*RV fragments (2.1 and 2.0 kbp) is revealed. For the transformed strain C180, a 0.9-kbp fragment is detected, as expected from the cleavage at the *Eco*RV site in the *URA3* sequence.

Strains C180 and C181 were both shown to require cysteine for growth. As expected, this nutritional requirement can be also satisfied by the addition of glutathione to the medium. In contrast, other organic sulfur compounds such as homocysteine, methionine, and *S*-adenosylmethionine do not sustain growth of these two strains. These results confirm that in *S. cerevisiae*, cysteine is derived from homocysteine only through the transsulfuration pathway. *S. cerevisiae* thus appears to be capable of incorporating inorganic reduced sulfur only into a four-carbon chain yielding homocysteine.

Transsulfuration enzymes are divided into two mutually exclusive classes. The sequences of cystathionine β-synthase and cystathionine γ-lyase from *S. cerevisiae* were compared

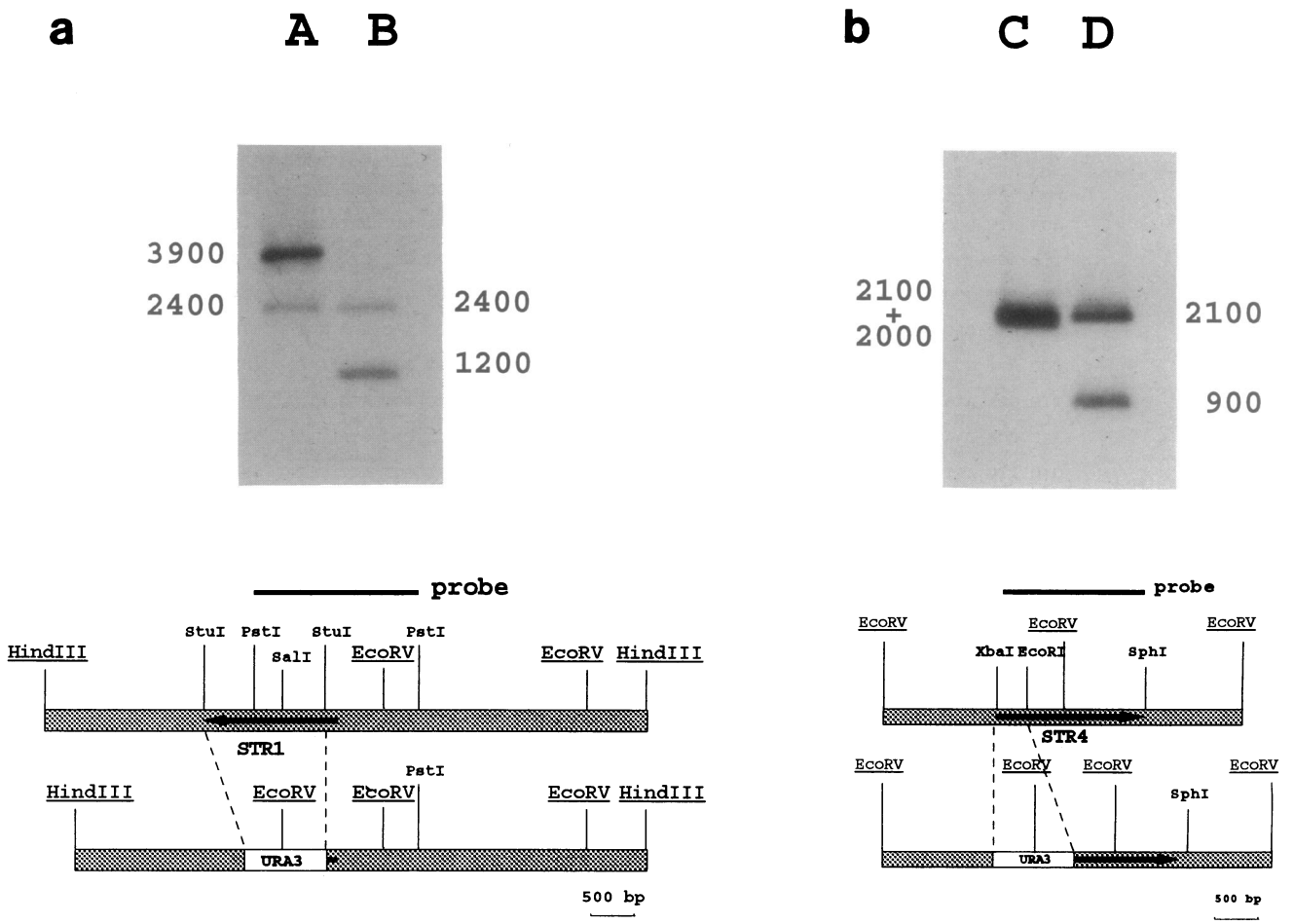

FIG. 4. (a) Southern blot analysis of *STR1* gene disruption. Lane A, *Eco*RV-*Hin*dIII digest of genomic DNA extracted from strain W303-1A; lane B, *Eco*RV-*Hin*dIII digest of genomic DNA extracted from strain C181. The filter was probed with the *Pst*I-*Pst*I fragment. (b) Southern blot analysis of the *STR4* gene disruption. Lane C, *Eco*RV digest of genomic DNA extracted from strain W303-1A; lane D, *Eco*RV digest of genomic DNA extracted from strain C180. The filter was probed with the *Xba*I-*Sph*I fragment.

against those in the National Biomedical Research Foundation sequence collection (version 74). Additional sequences were entered by us directly from the literature. Partial comparisons have already been published (9, 26, 28, 33). The sequences were aligned by using the Macaw program (29). Local alignments were optimized without creating additional gaps between the blocks of similarities. This search showed that transsulfuration enzymes can be partitioned into two exclusive families.

The first family is composed of the cystathionine β-synthases and cysteine synthases. Rat and yeast cystathionine β-synthases, 560 and 506 amino acid residues long, respectively, show extensive homology that encompasses the entire length of the yeast enzyme. The score of identity is 40% (199 identical residues). The rat enzyme has a 60-amino-acid long N-terminal extension, which accounts for the size difference between the two cystathionine β-synthases. These enzymes also exhibit extensive homology with the cysteine synthases from bacteria and plants (Fig. 5). For example, 120 amino acid residues are identical between the yeast cystathionine β-synthase and the cysteine synthase from spinach (37% identity with respect to the yeast enzyme), compared with the level of homology existing between the two cysteine synthases from *E. coli* (124 identical residues) or with that existing between the cysteine synthase from spinach and its *E. coli* counterpart encoded by the *cysK* gene (158 identical residues). It must be stressed that 59 amino acid residues (20%) are strictly conserved between the five enzymes of this family.

The second family includes the rat and yeast cystathionine γ-lyases, the yeast homocysteine synthase, and both the cystathionine γ-synthase and cystathionine β-lyase from *E. coli* (Fig. 6). The overall alignment reveals the presence of nine significant blocks of similarities which are dispersed along the polypeptides. The rat and yeast cystathionine γ-lyases are 47% conserved (170 identical residues). The yeast cystathionine γ-lyase also shows a high level of homology with the *E. coli* cystathionine β-lyase (94 identical residues) as well as with the *E. coli* cystathionine γ-synthase (128 identical residues). Similar levels of homology exist between the yeast homocysteine synthase and other members of this class; for example, yeast homocysteine synthase shares 99 and 104 identical amino acid residues with the *E. coli* cystathionine γ-synthase and the yeast cystathionine γ-lyase, respectively. However, the homocysteine synthase differs from the others by containing an insertion of about 30 amino acid residues in its middle part.

All of the transsulfuration enzymes utilize the same cofac-

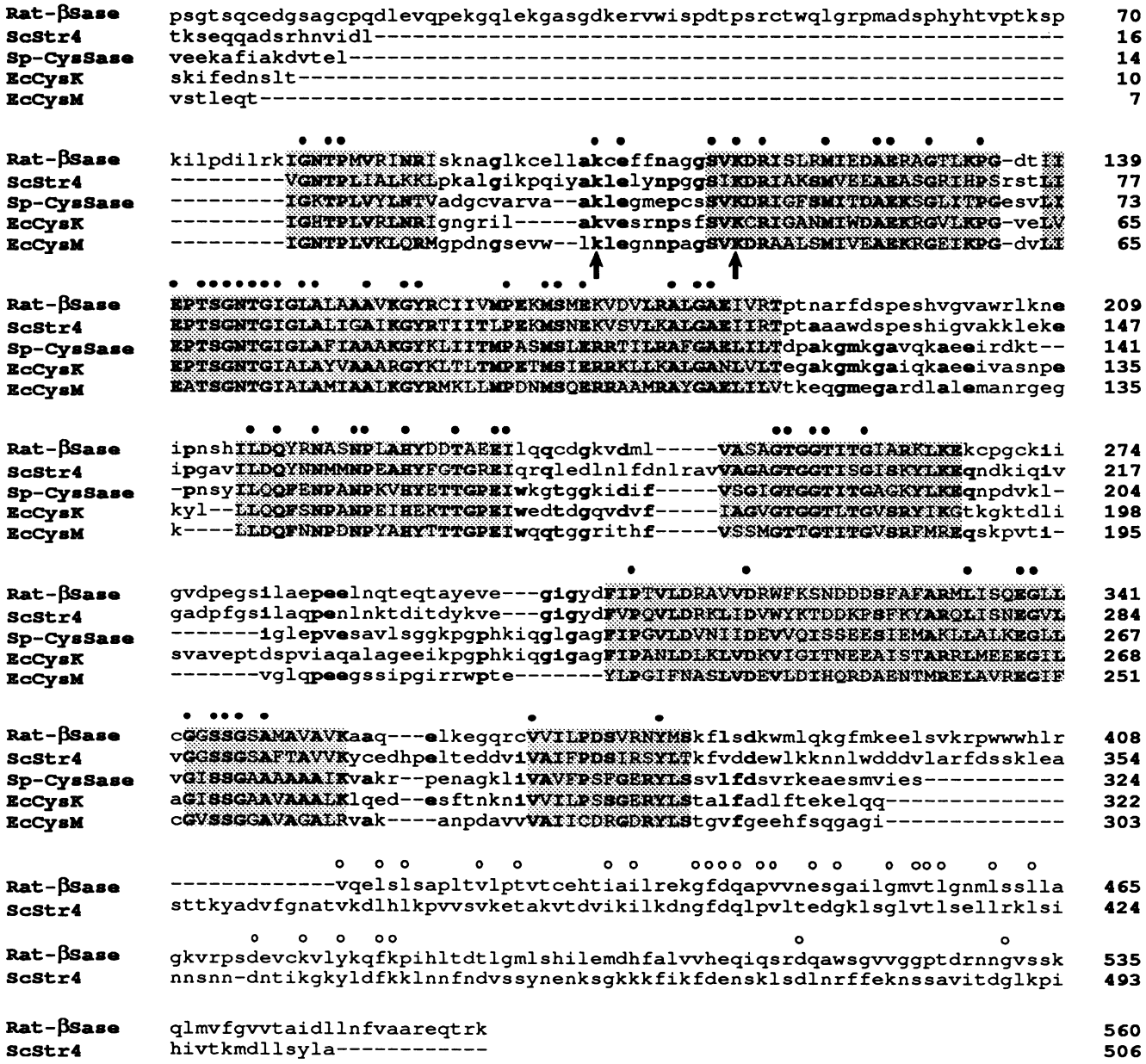

FIG. 5. Sequence alignments of cystathionine β-synthases and cysteine synthases. Rat-βSase, rat cystathionine β-synthase (33); ScStr4, cystathionine β-synthase encoded by the *S. cerevisiae STR4* gene; Sp-CysSase, spinach cysteine synthase (28); EcCysK, cysteine synthase encoded by the *E. coli cysK* gene (4, 19); EcCysM, cysteine synthase encoded by the *E. coli cysM* gene (32). The alignment was optimized by using the program of Schuler et al. (29). Blocks of similarities are in uppercase letters and boxed. Residues shared by at least three proteins are in bold letters. Black circles indicate residues strictly conserved in the five sequences. Open circles indicate the conserved residues of cystathionine β-synthases in the C-terminal extension. Possible pyridoxal binding sites are marked by arrows.

tor, pyridoxal phosphate, which forms a Schiff base with the ϵ-NH_2 moiety of a lysine residue. The lysine residues of rat and *E. coli* cystathionine γ-lyases identified as being bound to pyridoxal phosphate (22) are aligned with a lysine residue in the other proteins. This suggests that the pyridoxal phosphate cofactor of the cystathionine γ-lyase and homocysteine synthase from *S. cerevisiae* could be bound to the lysine residues indicated by an arrow in Fig. 6. By contrast, the pyridoxal phosphate binding site was identified neither for the cysteine synthases nor for the cystathionine β-synthases. However, the alignment depicted in Fig. 5 reveals that only two lysine residues were strictly conserved between all of these enzymes (corresponding to lysines 41 and 52 of the yeast cystathionine β-synthase). Thus, given the similarity of these enzymes, it is possible that one of these two lysine residues is the pyridoxal phosphate binding residue.

DISCUSSION

The yeast cystathionine β-synthase- and cystathionine γ-lyase-encoding genes were cloned by functional comple-

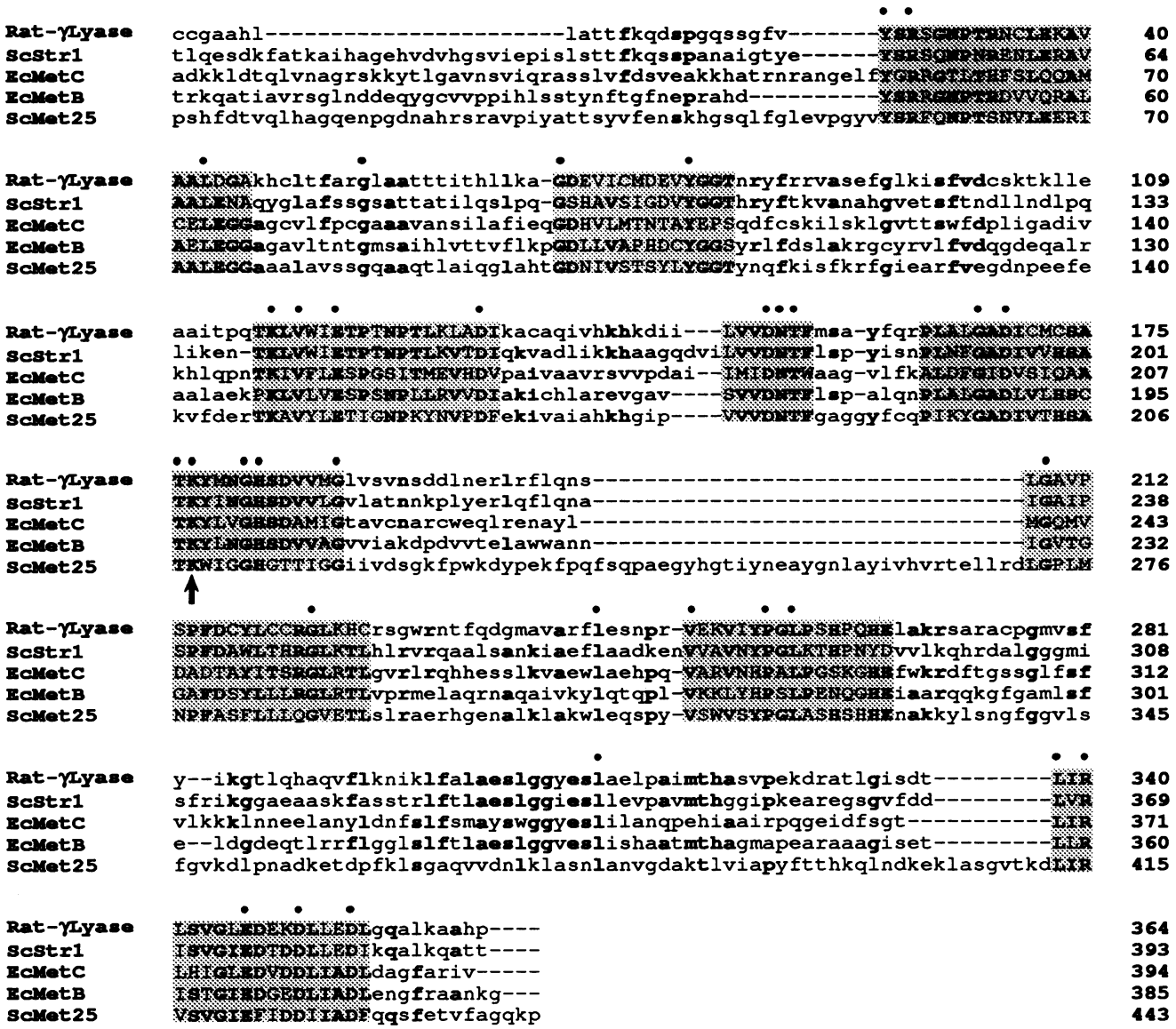

FIG. 6. Sequence alignments of cystathionine γ-lyases, cystathionine γ-synthase, cystathionine β-lyase, and homocysteine synthase. Rat-γLyase, rat cystathionine γ-lyase (9); ScStr1, cystathionine γ-lyase encoded by the *STR1* (*CYS3*) gene (24); EcMetC, cystathionine β-lyase encoded by the *metC* gene from *E. coli* (2); EcMetB, cystathionine γ-synthase encoded by the *metB* gene from *E. coli* (2); ScMet25, homocysteine synthase encoded by the *MET25* gene from *S. cerevisiae* (16). The alignment was optimized by using the program of Schuler et al. (29). Blocks of similarities are in uppercase letters and boxed. Residues shared by at least three proteins are in bold letters. Black circles indicate residues strictly conserved in the five sequences. The putative pyridoxal binding site is shown by an arrow.

mentation of strains bearing a mutated allele at the *STR1* or *STR4* gene. Disruptions of each of these genes gave viable haploid strains exhibiting a cysteine requirement. This auxotrophic requirement cannot be relieved by supplementation with other organic sulfur compounds, such as homocysteine and methionine. These results confirm our genetic analysis of sulfur metabolism in yeast cells (5): in *S. cerevisiae*, the sulfur atom resulting from the reduction of sulfate into sulfide is incorporated only in a four-carbon chain yielding homocysteine. Homocysteine is then used as a precursor for both cysteine and methionine synthesis. Sulfur metabolism in *S. cerevisiae* thus appears to be quite different from that existing in enterobacteria, in which the synthesis of cysteine is the first committed step of organic sulfur metabolism.

Systematic sequence comparison of enzymes catalyzing the transsulfuration steps, including those allowing the incorporation of inorganic sulfur into a carbon chain, revealed the existence of two separate groups. The first one contains cystathionine β-synthases and cysteine synthases, while the second one contains homocysteine synthase, cystathionine γ-synthase, cystathionine β-lyase, and cystathionine γ-lyases. This partition of transsulfuration enzymes, based on exclusive sets of sequence motifs, appears to be mainly correlated at the functional level with the length of the carbon chain of the amino acid substrate. Indeed, the first family is exclusively composed of enzymes catalyzing the β-replacement reactions of three-carbon-chain amino acids. The second family appears to be more heterogeneous. It is

composed of enzymes catalyzing γ-replacement reactions of four-carbon-chain amino acids and the γ cleavage of cystathionine. However, this family comprises also the enzyme catalyzing the β cleavage of cystathionine. The presence of cystathionine β-lyase in this second family could reflect the history of the emergence of this enzyme (see below). The strong similarity displayed by the members of each family suggests that all transsulfuration enzymes may have evolved from only two ancestral proteins which could have been pyridoxal phosphate enzymes. Considering the alignment of cysteine synthases and cystathionine β-synthases, it seems reasonable to speculate that the latter could have been achieved by the fusion of an additional C-terminal domain to a primitive cysteine synthase. This specialization could have been favored by substrate ambiguity of such a primitive cysteine synthase. Accordingly, it must be stressed that substrate ambiguity remains in modern cysteine synthases. For example, the *E. coli cysM*-encoded product was shown to be capable of catalyzing both cysteine and *S*-sulfocysteine synthesis (17). The data presented are also consistent with a model in which the common ancestor of the second group of transsulfuration enzymes could have been a primitive homocysteine synthase. Traces of the broad reactivity of such an ancestor homocysteine synthase emerge in contemporary metabolism. The modern homocysteine synthase is indeed capable of catalyzing in vitro different reactions, such as sulfhydrylation of *O*-acetylserine (37). In vivo studies have also demonstrated the capacity of the yeast homocysteine synthase to catalyze the conversion of ethionine into methionine (6).

According to these speculative statements, direct incorporation of sulfide into both a three- and a four-carbon chain must have coexisted in primitive organisms. The building up of the transsulfuration pathway(s) bridging the biosyntheses of cysteine and homocysteine could have been subsequently achieved by the enzymatic recruitment of cysteine synthase and homocysteine synthase. As indicated by the overall similarities exhibited by the transsulfuration enzymes, such transitions to new catalytic functions could occur through only a few genetic changes. This evolution could have been retained especially to favor the coordinate control of sulfur amino acid biosynthesis. It is worth stressing that the hypothesis of the coexistence of ancient cysteine and homocysteine synthases is strongly reinforced by experimental data acquired on sulfur metabolism in enterobacteriaceae; for example, Simon and Hong (31) have discovered the latent capacity of *E. coli* to synthesize homocysteine from *O*-succinylhomoserine, bypassing the cystathionine pathway. This reaction was found to occur in a methionine prototroph revertant of a cystathionine γ-synthase-deficient strain. This new mutation defines a gene, *metQ*, encoding a 41-kDa polypeptide (23) that could be a homocysteine synthase.

Other constraints seem to have been imposed on the evolution of this family of proteins, perhaps to meet the starvation experienced by all microorganisms in natural environments. Indeed, yeast cystathionine γ-lyase is completely devoid of cysteine residues, and cystathionine β-synthase of the same organism contains only one cysteine residue. By contrast, the rat cystathionine γ-lyase and cystathionine β-synthase contain 14 and 13 cysteine residues, respectively. This observation could be related to the fact that in *S. cerevisiae*, the cysteine content strictly depends on the reactions catalyzed by these enzymes, whereas in mammals, cysteine is provided directly by the diet.

ACKNOWLEDGMENTS

We are indebted to G. N. Cohen for critical reading of the manuscript.

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