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S-Adenosyl Methionine-Mediated Repression of Methionine Biosynthetic Enzymes in *Saccharomyces cerevisiae*

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S-adenosylmethionine (SAM) has been shown to provoke repression of some methionine-specific enzymes in wild-type cells, namely, adenosine triphosphate sulfurylase, sulfite reductase, and homocysteine synthetase. Repressive effects observed in SAM-supplemented cultures should be due to SAM per se, since the intracellular pool of SAM increases while the intracellular pool of methionine remains low and constant. Derepression brought about by methionine limitation is accompanied by a severe decrease in SAM as well as methionine pool sizes, although methionine adenosyl transferase is slightly derepressed. Different hypotheses have been considered to account for the previously reported implication of methionyl transfer ribonucleic acid and the presently reported SAM effects in this regulatory process.

Studies on regulatory aspects of methionine biosynthesis in *Saccharomyces cerevisiae* have shown that exogenous methionine exerts a potent repression on the synthesis of some enzymes involved in this pathway which have been called met group I enzymes (4, 5). Different hypotheses were made concerning the nature of the repressor-corepressor system. Besides free methionine, the two activated forms of this amino acid, i.e., methionyl-transfer ribonucleic acid (tRNA) and *S*-adenosyl methionine (SAM) were postulated as likely candidates for such a role (4). Further studies have lent support to the participation of methionyl-tRNA as a regulatory signal in methionine biosynthesis in *S. cerevisiae*. This conclusion was reached from results obtained with a thermosensitive mutant (5) bearing an impaired methionyl-tRNA synthetase (L-methionine: soluble RNA [sRNA] [adenosine monophosphate], EC 6.1.1.10) and results obtained in methionine limitation with a methionine auxotroph (25). In both sets of experiments, a correlation was established between the level of met-tRNA^{met} per cell and the rate of synthesis of met group I enzymes. Nevertheless, these results did not rule out a role for SAM in this regulatory process.

In fact, results obtained with *Escherichia coli* implicated SAM as a participant to the regulation of methionine biosynthesis, since *metK* mutants, synthesizing low levels of methionine adenosyl transferase (adenosine triphosphate [ATP]: L-methionine *S*-adenosyl transferase, EC 2.5.1.6) have elevated levels of three enzymes involved in this pathway (10, 18, 24). However, in *Salmonella typhimurium*, *metK* mutants, although nonrepressible for methionine biosynthetic enzymes (13, 17), have a wild-type level of methionine adenosyl transferase (18). In addition, methionine adenosyl transferase itself is subject to regulation of its synthesis, as shown by repression in methionine-supplemented cultures and by study of *metJ* regulatory mutants of *E. coli* (11) and of *S. typhimurium* (18).

Results presented here show that, in *S. cerevisiae*, besides methionyl-tRNA, SAM is also implicated in the regulation of some methionine biosynthetic enzymes.

MATERIALS AND METHODS

Strains. The haploid strains of *S. cerevisiae* used for this investigation were 4094-B (*α, ade2, ura1*; from F. Sherman, Rochester, N.Y.) and D6 (*α, met2, ura*; from M. Grenson, Brussels, Belgium).

Cultures. The minimal medium and culture techniques were as described by Cherest et al. (4). The concentrations of methionine and SAM used are given in the text. The chemostat conditions have been described previously (5, 25). Cells collected by centrifugation and washed were immediately used either for

preparation of crude extracts or for extraction of pools.

Extracts. The cell extracts were made as described previously (5). The crude extract was centrifuged for 90 min at 40,000 rpm in a rotor 40 of a preparative Spinco centrifuge (S-40 extract). This lowers the blanks in the ATP sulfurylase assay.

Enzyme assays. ATP sulfurylase (ATP:sulfate adenylyl transferase, EC 2.7.7.4) activity was measured in the S-40 extract by the method of Wilson and Bandurski (27) as described by de Vito and Dreyfuss (7). Homocysteine synthetase (26) activity was assayed in the Spinco extract as described by Wiebers and Garner (26) at 30 C, and the homocysteine formed was estimated by the method of Kredich and Tomkins (12). The sulfite reductase (hydrogen sulfide: nicotinamide adenine dinucleotide phosphate oxidoreductase, EC 1.8.1.2) activity was measured in crude extract kept at room temperature and assayed within 1 h after extraction, by the method of de Vito and Dreyfuss (7). The sulfide formed was estimated by the method of Siegel (22).

Methionine adenosyl-transferase activity was measured in the crude extract as follows. The incubation mixture contained, in 1 ml: tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.5, 100 μmol; $MgCl_2$, 100 μmol; KCl, 100 μmol; reduced glutathione, 20 μmol; ATP, 30 μmol; L-methionine, 20 μmol; and crude extract containing 4 mg of protein. A blank without methionine was employed for each assay. The assays were incubated for 1 h at 37 C; the reaction was stopped by 1 ml of 1.5 N perchloric acid, and the precipitated proteins were removed by centrifugation. The SAM formed by the reaction was isolated on Dowex 50W X8 (100 to 200 mesh) in the H^+ form by the column procedure of Schlenk and de Palma (20). The fractions containing SAM were pooled, their volume and optical density at 256 nm (OD_{256nm}^{1cm}) were measured and the SAM concentration was calculated with the use of E_M (256 nm) = 14,700 (21).

Protein concentration. The protein estimation was performed by the biuret method (9) with bovine serum albumin as reference.

Specific activities. Specific activities are expressed in nanomoles per minute per milligram of protein, i.e., 10^{-3} international units.

Determination of the amount of methionyl-tRNA^{met} and tRNA^{met}. The extraction of tRNAs and the determination of the amount of tRNA^{met} charged in vivo were as previously described (25). For tRNA^{met} determination, tRNAs previously deacylated were charged with the use of yeast methionyl-tRNA synthetase (25) for 10-min incubation periods; single and double doses of tRNA were used to ensure that tRNA^{met} was always limiting and in the proportional range of concentrations. Results are expressed as nanomoles of L-methionine charged per minute and milligram of tRNA. A solution of 1 mg of tRNA/ml yields an OD_{260nm}^{1cm} value of 22.

Determination of the intracellular SAM pool. The SAM was estimated, in a perchloric extract of the cells (see below), by chromatography on Dowex 40 in the Na^+ form as described by Shapiro and Ehninger (21). The cells were centrifuged, washed with cold water, and suspended in three times their volume of 1.5 N perchloric acid. After 1 h at 4 C, this suspension was centrifuged (12,000 × *g*, 10 min) and the clear supernatant fluid was neutralized to pH 6 to 7 with a 2 M $KHCO_3$ solution. The mixture was then centrifuged and a sample (generally 5 ml) was adsorbed on a column (1 cm in diameter by 4 cm) of Dowex 50W x8 (100 to 200 mesh) in the Na^+ form. It was washed with 0.1 M NaCl until the OD_{256nm}^{1cm} was less than 0.050 (100 ml was generally enough) and SAM was eluted with 6 N H_2SO_4 (generally 60 ml was sufficient). The tubes containing SAM were pooled and the SAM concentration was calculated as mentioned above.

Determination of the intracellular pool of methionine. A 10-ml amount of sterile water was added to 2 ml of packed cells; the resulting suspension was boiled for 10 min in a water bath and then centrifuged. The concentration of L-methionine in the supernatant fluid was determined with *Leuconostoc mesenteroides* P-60 ATCC 8042 by use of the Difco methionine assay medium and as described in the Difco Manual. A standard curve was constructed with a known solution of L-methionine and repeated for each series of assays. Suitable samples of the above supernatant fluid were used so that their methionine content falls into the linear part of the standard curve.

Chemicals. SAM was purchased from Sigma Chemical Co. Dowex 50-W X8 (100 to 200 mesh) was purchased from Lambert-Rivière (France) and washed prior to use as described by Shapiro and Ehninger (21). It is brought either to H^+ form or to Na^+ form as described by these authors. *S*-adenosyl-L [Me-^{3}H] methionine was purchased from Commissariat à l'Energie Atomique (France).

RESULTS

Repression effects of SAM in a wild-type strain. Although the studies of *metK* mutants in *E. coli* pointed to the role of SAM in the regulatory mechanism, direct evidence that SAM behaved as an exogenous repressor could not be obtained since there is no uptake of this compound in this organism (11). On the contrary, in *S. cerevisiae*, Murphy and Spence (15) showed that SAM is actively concentrated by a specific uptake system. It was then possible to search for a role of exogenous SAM in the synthesis of three methionine group I enzymes, i.e., homocysteine synthetase, ATP sulfurylase, and sulfite reductase.

Increasing concentrations of SAM were used (Table 1). Sulfite reductase and ATP sulfurylase, which were nearly 100% repressible when exogenous methionine was used, were as efficiently repressed by SAM, whereas homocysteine synthetase was only 80% repressible by SAM as well as by methionine. In addition, as reported previously in the case of methionine (5), a very sharp response to the exogenous

concentration of SAM occurred. At 0.05 mM SAM, very little repression was obtained, but a twofold increase of this concentration repressed homocysteine synthetase and ATP sulfurylase maximally.

Evidence that SAM is directly implicated in repression. Since SAM seems to mimic the repression effects of exogenous methionine, the question was raised whether SAM was acting per se or by its transformation into free methionine. To answer this question, the sizes of the intracellular pools of these two compounds were determined. For this purpose, cells were grown in the presence of concentrations of SAM identical to those used for the repression studies. Results in Fig. 1 show that the endogenous concentration of SAM increased sharply with increasing exogenous concentration of this compound, whereas the free methionine pool remained remarkably constant and comparable to that observed in cells grown in minimal medium. Since the maximal attainable repression was obtained at 0.15 mM exogenous SAM, one would expect that endogenous methionine biosynthesis was stopped. Results from experiments with $^{35}SO_4^{2-}$ used under identical conditions agree with this hypothesis, since the amount of methionine biosynthesized by the cells was reduced by 96% after 6 h of growth in the presence of 0.15 mM exogenous SAM (J. Antoniewski and H. de Robichon-Szulmajster, unpublished data). It follows that, at least at this SAM concentration (0.15 mM), the free methionine found in the pool has to be derived from SAM. Cultures grown in the presence of SAM [Me-^{3}H] have shown that this is the case. Under conditions where SAM represses, the free methionine pool remained at the same level as in unrepressed cells. This excludes the possibility that the observed repression could be due to methionine per se. Thus, a direct effect of SAM, or a derivative of it, is indicated.

Pools of free methionine and SAM after growth in the presence of methionine. It is well known that, in wild-type cells of *S. cerevisiae*, methionine is efficiently converted into SAM (19). One could then ask whether the repressive effects of exogenous methionine could be related to its conversion into SAM. Pool sizes for these two components have been measured in cells grown at different exogenous concentrations of methionine. Results of a typical experiment are reported in Table 2. It can be seen that, in mimimal medium, as shown above, the methionine and SAM pool sizes are of the same order of magnitude. At the lowest methionine concentration used, 0.2 mM, which leads to a partial repression of the methionine group I enzymes (see Table 1 and reference 5), both pools were only slightly elevated. At higher concentrations, methionine was actively concentrated and, concomitantly, SAM accumulated. Hence, these data are roughly compatible with the idea that repressive effects of exogenous methionine could be due to its transformation into SAM. However, comparison of data from Tables 1 and 2 and Figure 1 do not

TABLE 1. *Effect of various concentrations of S-adenosyl methionine (SAM) and methionine on synthesis of some methionine group I enzymes in a wild-type strain (4094-B)*

Addition to minimal medium	Specific activity[a]		
	Homocysteine synthetase	ATP sulfurylase	Sulfite reductase
None	340	106	1.82
DL-Methionine			
0.2 mM	150	30	0.56
2 mM	75	7	0.07
20 mM	80	7	—
L-SAM			
0.015 mM	320	93	—
0.05 mM	248	81	—
0.1 mM	90	6	—
0.15 mM	60	6	0
0.6 mM	80	0	—

[a] Expressed in nanomoles per minute per milligram of protein.

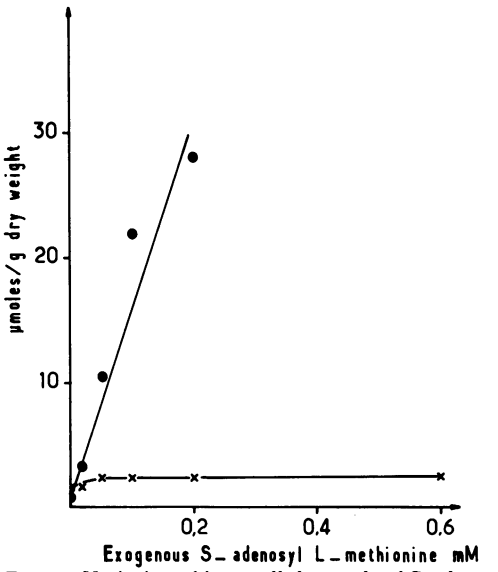

FIG. 1. *Variation of intracellular pools of S-adenosyl methionine (SAM) and free methionine in wild-type cells (4094-B) grown in the presence of various concentrations of SAM.* (●) *SAM pool.* (×) *Methionine pool.*

TABLE 2. *Pools of free methionine and S-adenosyl methionine (SAM) in wild-type cells (4094-B) grown in different concentrations of exogenous methionine*

Addition to minimal medium	Methionine pool[a]	SAM pool[a]
None	1.5	1.1
DL-Methionine		
0.2 mM	3	2.5
2 mM	55	12
20 mM	80	11

[a] Expressed in micromoles per gram (dry weight).

fully agree with this hypothesis. For example, if one considers the effects of 0.2 mM exogenous DL-methionine, it can be seen that a marked repression of enzyme synthesis has already occurred (see Table 1) while low values of methionine and SAM pools are still observed (Table 2). When an identical SAM pool was formed from exogenously added SAM, no repression occurred (Fig. 1 and Table 1). It must be emphasized, however, that the extrapolation of the results obtained when SAM is exogenously supplied to the situation which exists in cells where SAM is synthesized endogenously from methionine is not necessarily justifiable. For example, biosynthesized SAM and exogenously added SAM might not reach the site where they can exert their repressive effect with the same efficiency.

Relation between the pool size of SAM and enzymatic derepression. Since the addition of exogenous methionine is accompanied by accumulation of SAM in the wild-type cells under conditions which also lead to repression of methionine group I enzymes, it was of interest to determine the effect of methionine limitation on the size of SAM pools. For this purpose, we have used a methionine auxotroph, strain D6. This strain was previously found to be devoid of homoserine-*O*-transacetylase activity (4) and then to be able to grow in the presence of *O*-acetyl-homoserine, a methionine precursor. Since growth of a wild-type strain in *O*-acetyl-homoserine-supplemented medium does not lead to any repressive effect on homocysteine synthetase synthesis, such a medium was used to ensure growth of the "control" culture, i.e., the culture used to inoculate the chemostat. The "control" then exhibits intermediate levels of methionine biosynthetic enzymes from which the range of repression and derepression for any of these enzymes can be determined. The values reported in Table 3, from the "control" culture of strain D6 are not similar to those reported from culture in minimal medium of the wild-type strain 4094-B, especially with regard to the specific activity of homocysteine synthetase and to the pool size of SAM. This is not surprising since such differences are frequently observed between strains of widely different origin such as strains D6 and 4094-B.

Results in Table 3 show that, as soon as methionine limitation was effective (in view of the methionine pool size, first fraction of the chemostat), a large drop in SAM pool occurred and synthesis of a typical group I enzyme, homocysteine synthetase, appeared to be derepressed. In further fractions, both methionine and SAM pool sizes remained quite constant, while derepression of homocysteine synthetase synthesis continued. This probably indicates that, whatever is the final repressor, its concentration decreases more slowly than the methionine and SAM pools. Nevertheless, these results are compatible with a correlation between derepression of methionine group I enzymes and pool sizes of both SAM and methionine.

The lowering in the SAM pool size in the chemostat fractions cannot be due to a decrease in methionine adenosyl transferase activity since its synthesis appeared rather derepressed. Moreover, in excess methionine, the synthesis of this enzyme appeared to be repressed, results which do not agree with those previously reported by Pigg et al. (16), but which do confirm those recently published by Mertz and Spence (14). Our results then show that, in *S. cerevisiae*, both methionine-activating enzymes, methionyl-tRNA synthetase (25) and methionine adenosyl transferase, are under repressive control exerted by their common substrate, L-

TABLE 3. *Study of a methionine auxotroph (D6) in excess methionine and methionine limitation*

Growth conditions[a]	Homocysteine synthetase[b]	SAM synthetase[b]	Methionine pool[c]	SAM pool[c]
Repression	27	0.53	5.7	34
Control	61	1.3	0.9	7.0
Chemostat				
F I	226	3.9	0.5	1.57
F II	336	4.0	0.3	1.08
F III	390	7.1	0.3	1.15
F IV	420	—	0.4	0.85

[a] Repression: cells grown in 2 mM DL-methionine. Control: cells grown in 0.5 mM *O*-acetyl-DL-homoserine; a sample of these cells was used to inoculate the chemostat. Chemostat: as described previously (5, 25); F I to IV correspond to successive fractions of the chemostat.

[b] Enzymatic activities are expressed in nanomoles per minute per milligram of protein.

[c] Pools are expressed in micromoles per gram (dry weight).

methionine, in agreement with observations in *E. coli* (1, 11).

Effect of exogenously added methionine and SAM over tRNA^{met} content and in vivo charging. Results presented above have shown that methionine limitation leads to a decreased SAM pool and to derepressed synthesis of methionine group I enzymes. On another hand, it has been shown previously that, under identical conditions, the methionyl-tRNA^{met} content per cell could be related to the rate of synthesis of these enzymes (25). Since, as presently shown, SAM can promote repression of methionine group I enzymes, it was thought of interest to investigate the possible effects of SAM over synthesis and in vivo charging of tRNA^{met}. For this purpose, a wild-type strain was grown in minimal medium and in medium supplemented either by 0.2 mM L-SAM or by 20 mM DL-methionine.

Results in Table 4 show that, as previously demonstrated with another wild-type strain, the tRNA^{met} is already 100% acylated after growth in minimal medium (5). As expected, excess of methionine or SAM did not modify this in vivo charging. Although a change in the total amount of tRNA^{met} per cell could have been conceivable in one or the other case, results obtained show no apparent difference among the three cultures. However, these findings do not exclude a possible effect of SAM over tRNA^{met} synthesis, if one assumes, as previously mentioned (5), that only a minor subspecies of tRNA^{met} acts as the regulatory signal.

DISCUSSION

Results presented here show beyond any doubt that SAM is implicated in regulation of methionine group I enzyme synthesis in *S. cerevisiae*. The strongest evidence comes from SAM-supplemented cultures of wild-type cells in which full repression occurs when the pool size of SAM is greatly enhanced while the pool size of methionine remains unchanged. Other results are compatible with the implication of SAM in the regulatory process: (i) the SAM pool size sharply decreases during derepressed synthesis of methionine group I enzymes in methionine limitation as well as the methionine pool size itself, and (ii) the pool size of SAM increases concomitantly with that of free methionine in cells grown in the presence of various exogenous methionine concentrations, in which repression is observed. It was shown before that methionyl-tRNA rather than free methionine is involved in this regulatory process (5, 25). Indeed, the regulatory effect of SAM could be separated from the methionyl-tRNA^{met} effects. Two distinct methionine repressors could be made either acting independently at the same regulatory site, or acting at two regulatory levels, i.e., transcription and translation.

TABLE 4. *Effect of exogenously added methionine and S-adenosyl methionine (SAM) over tRNA^{met} content and in vivo charging*[a]

Addition to minimal medium	tRNA^{met}[b]	tRNA^{met} charged in vivo (%)[c]
None	0.085	100
DL-Methionine, 20 mM	0.063	99
L-SAM, 0.2 mM	0.080	92

[a] Strain 4094-B.

[b] Expressed as nanomoles of L-methionine charged per minute per milligram of tRNA (see Materials and Methods).

[c] Ratio of the acceptor activity of periodate treated over the acceptor activity of untreated tRNA × 100 (see also reference 25).

However, an alternative hypothesis can be made on the basis of metabolic connections between SAM and tRNAs. Firstly, it is well established in bacteria that SAM is, together with ornithine, one of the precursors of polyamine biosynthesis, which appears to regulate the rate of tRNA synthesis (6, 8). Secondly, SAM, the only biological methylating agent, can influence the methylation pattern of tRNAs (3). Regulatory mutants in the histidine biosynthetic pathway in *S. typhimurium* have already been related to modifications of tRNA^{his} content and to the absence of a specific substituent in tRNA^{his} (2, 23). It is then conceivable that SAM effects uncovered here could be attributed either to an indirect modification of the rate of tRNA synthesis, or to the modification of specific methylated nucleosides, important for methionyl-tRNA^{met} to exert its regulatory effect (or both). If this hypothesis is correct, one would expect that the level of SAM would affect the regulation of, at least, some other biosynthetic pathways in which acyl tRNAs are implicated as regulators. This assumption has not been tested.

In any case, results obtained upon methionine limitation showing both a decrease in methionyl-tRNA^{met} cellular content (25) and a decrease in SAM present in the pool (present paper) could fit with the alternative hypothesis, since the lowering in SAM endogenous concentration could be the cause of the reduced tRNA synthesis observed. Yet, the presence of exogenously added SAM did not influence either the amount or the in vivo charging of tRNA^{met}

in cultures of a wild-type strain. It remains then possible that only a minor subspecies of $tRNA^{met}$ is involved in this regulatory process.

At the present time, the experimental evidence is not sufficient to exclude one or the other hypothesis.

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