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Methionine- and S-Adenosyl Methionine-Mediated Repression in a Methionyl-Transfer Ribonucleic Acid Synthetase Mutant of *Saccharomyces cerevisiae*[1]

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A *Saccharomyces cerevisiae* mutant strain unable to grow at 38 C and bearing a modified methionyl-transfer ribonucleic acid (tRNA) synthetase has been studied. It has been shown that, in this mutant, the percentage of $tRNA^{met}$ charged in vivo paralleled the degree of repressibility of methionine biosynthetic enzymes by exogenous methionine. On the contrary, the repression mediated by exogenous S-adenosylmethionine does not correlate with complete acylation of $tRNA^{met}$. Although McLaughlin and Hartwell reported previously that the thermosensitivity and the defect in the methionyl-tRNA synthetase were due to the same genetic lesion (1969), no difference could be found in the methionyl-tRNA synthetase activity or in the pattern of repressibility of methionine biosynthetic pathway after growth at the premissive and at a semipermissive temperature. It appears that the mutant also exhibits some other modified characters that render unlikely the existence of only one genetic lesion in this strain. A genetic study of this mutant was undertaken which led to the conclusion that the thermosensitivity and the other defects are not related to the methionyl-tRNA synthetase modification. It was shown that the modified repressibility of methionine biosynthetic enzymes by methionine and the lack of acylation of $tRNA^{met}$ in vivo follow the methionyl-tRNA synthetase modification. These results are in favor of the idea that methionyl-$tRNA^{met}$, more likely than methionine, is implicated in the regulation of the biosynthesis of methionine.

If we consider the regulation of the methionine biosynthetic pathway, somewhat divergent results appear from studies of various microorganisms. Strong evidence has been presented that S-adenosylmethionine (SAM) rather than methionine is involved as a regulatory effector in *Escherichia coli* and *Salmonella typhimurium* (11, 12, 15). Moreover, data so far obtained with *S. typhimurium* by Gross and Rowbury (13, 14) and with *E. coli* by Ahmed (1) led these authors to eliminate methionyl-$tRNA^{met}$ (met-$tRNA^{met}$) as another possible co-repressor. This conclusion was provided by the fact that *metG* mutants defective in methionyl-tRNA synthetase (MTS) have been found to have wild-type levels of cystathionine gamma synthetase when grown with a low concentration of methionine and to be repressible by methionine. According to Paszewski and Grabski (20), it seems that, in *Aspergillus nidulans*, regulation of methionine biosynthesis involves neither methionine nor SAM but more likely cysteine and/or perhaps homocysteine. In *Saccharomyces cerevisiae* we have already reported that both activated products of methionine, SAM and met-$tRNA^{met}$, are involved separately in the regulation of synthesis of this amino acid (2, 4). As far as SAM is concerned, similar results have been found by Ferro and Spence (8). The implication of met-$tRNA^{met}$ was evidenced in our laboratory (4) from results provided by the study of a mutant isolated by McLaughlin and Hartwell and reported by these authors to be devoid of MTS activity in vitro (19). It was found that in this mutant, H.19.3.4 (locus *MES1*), the synthesis of at least four enzymes involved in methionine biosynthesis and designated as met group I enzymes was not repressed in the presence of 0.2 mM DL-methionine in the culture medium, whereas under the same growth conditions a large amount of repression of met group I enzymes synthesis was already obtained in a wild-type strain (4). In addition, it appeared that the non-repressibility of met group I enzymes in the

[1] We hope this paper will honor the memory of Dr. Huguette de Robichon-Szulmajster, who died prematurely in April 1974.

mutant strain was accompanied by a low level of in vivo $tRNA^{met}$ charging. Moreover, growth of the mutant in the presence of a high methionine concentration (such as 4 mM) led to the recovery of both repressibility and complete in vivo $tRNA^{met}$ charging (4).

In view of further results showing the role of SAM as a regulatory signal in a wild-type strain of *S. cerevisiae*, as well as in some mutants exhibiting a loss of sensitivity towards methionine-mediated repression (3), it was of interest to examine the possible action of SAM on the regulation of methionine biosynthesis in the mutant carrying an impaired MTS activity.

During the course of this study, it appeared that strain H.19.3.4 displays various phenotypic characters that cannot be explained on the basis of only one genetic lesion. In fact, the original mutant strain was isolated by McLaughlin and Hartwell as a thermosensitive mutant unable to grow at 38 C and was found to exhibit a methionine requirement that does not result from the loss of activity of any methionine biosynthetic enzyme. From their study of strain H.19.3.4, McLaughlin and Hartwell concluded that both the thermosensitivity and the methionine requirement are directly related to the mutation leading to the existence of an impaired MTS (19). It will be shown in this paper that such an interpretation was not correct since we have been able to separate the mutation responsible for the modified MTS from the other defects. Moreover, the present study provides additional evidence that exogenous SAM and exogenous methionine act separately on the regulation of met group I enzymes synthesis.

MATERIAL AND METHODS

Strains. The haploid strains of *S. cerevisiae* used for this investigation were the following: 4094-B (*α*, *ade2*, *ura1*) from F. Sherman's collection and H.19.3.4 (*a*, *ade1*, *leu2*, *lys11*, *his4*, *his5*, *mes1*) from L. H. Hartwell's collection.

Genetics techniques. The following diploid strain was made: CC196 = H.19.3.4 × 4094-B. One of the tetrads of this cross was extensively studied: CC196-6. Sporulation of the diploid was induced by the method of McClary et al. (18). Ascospores were isolated as described by Johnston and Mortimer (16).

Growth curves. Growth was in Erlenmeyer flasks and determined as described previously (5).

Cultures. All cultures were grown overnight at 28 C with agitation and harvested in the middle of the exponential phase of growth, i.e., an optical density (1 cm, 650 nm) of 1.5 measured in a Zeiss PM QII spectrophotometer. The minimal medium used for 4094-B strain was GO medium (9) supplemented with uracil (10 mg/liter) and adenine (10 mg/liter). For strain H.19.3.4 and for segregants of tetrads CC196-6, this medium received the addition of DL-leucine, DL-lysine, and DL-histidine (100 mg/liter). Methionine and SAM concentrations (where appropriate) are given in the text or the tables.

Determination of pools. Methionine pools were determined by using *Leuconostoc mesenteroides* as described previously (2, 3). SAM pools were estimated as already reported (2, 3). All pools were expressed as micromoles of methionine or SAM per gram (dry weight).

Extracts. For homocysteine synthetase and adenosine triphosphate (ATP) sulfurylase (ATP:sulfate adenylyl transferase, EC 2.7.7.4), extracts were made as described previously (4), but the crude extract was then centrifuged for 90 min at 100,000 × *g* (S100 extract), which lowers the blanks in the ATP sulfurylase assay. Buffers used for the extraction of these two enzymes were given in the same paper (4).

For MTS (L-methionine:soluble RNA ligase [adenosine monophosphate], EC 6.1.1.10), the extracts were made in 20 mM potassium phosphate buffer (pH 7.5) containing 5 mM 2-mercaptoethanol and 20% glycerol (wt/vol). The extracts were centrifuged for 90 min at 100,000 × *g* and then dialyzed overnight against the extraction buffer.

Enzymatic assays. Homocysteine synthetase activity was assayed in the S100 extract as described by Wiebers and Garner (24) at 30 C, and the homocysteine formed was estimated by the method of Kredich and Tomkins (17). ATP sulfurylase activity was also measured in the S100 extract by the method of Wilson and Bandurski (25) as described by de Vito and Dreyfuss (6). The measurement of activity of MTS was as described previously (3). For K_m determinations, the range of substrate concentrations used was 0.0005 to 1 mM for methionine, 0.05 to 10 mM for ATP, and 0.05 to 10 mg/ml for tRNA; when one substrate was varied, the two others were kept constant and at the concentration given in the assay procedure (3).

Protein concentration. The protein estimation was performed by the biuret method (10) with bovine serum albumin as reference.

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

Extraction of tRNA's and determination of the amount of met-$tRNA^{met}$. Extraction of tRNA's and determination of the amount of met-$tRNA^{met}$ were performed by a modification of a previously described procedure (23). First, according to Ehresman et al. (7), the best reproducibility in the determination of the in vivo level of tRNA charging in yeast was obtained if the tRNA's were extracted from cells killed by sodium azide. Therefore, to a 1-liter culture (optical density of 2 at 650 nm and a 1-cm light path) was added 10 ml of 0.1 M sodium azide. The cells were then rapidly cooled by mixing with crushed ice, centrifuged, and suspended in 25 ml of distilled water. A 0.25-ml amount of a 3% solution of sodium dodecyl sulfate was added, followed by 25 ml of an 80% solution of phenol in water (wt/wt); we also added 100 mg of macaloid to inactivate

most of ribonuclease activities. The mixture was shaken vigorously for 1 h at room temperature (about 23 C). The two phases were separated by centrifugation at 6,000 × *g* for 15 min. The aqueous layer was washed twice with 50 ml of cold ether containing 5 ml of 1 M sodium acetate (pH 4.5). A 50-ml amount of distilled ethyl alcohol (−15 C) was then added, and the RNA was allowed to precipitate for at least 30 min at −15 C. All further operations, i.e., periodate oxidation, stripping of tRNA's, and determination of their acceptor activity, were performed as described previously (4). The only exception resides in the time period of the incubation leading to the determination of $tRNA^{met}$ acceptor activity, which was of 30 min instead of 10 min. Results are expressed as a percentage of tRNA charged, i.e., the ratio of the acceptor activity of periodate treated to acceptor activity of the untreated tRNA × 100.

Chemicals. *O*-acetyl-DL-homoserine was synthesized for us by M. Cherest by the method of Sakami and Toennies (21). ^{14}COOH-L-methionine (49 mCi/mmol) was purchased from C.E.A., France. Sodium dodecyl sulfate was recrystallized in ethyl alcohol. Commercial yeast tRNA was purchased from Schwarz Bio Research. *S*-adenosylmethionine chloride, grade II, was obtained from Sigma Chemical Co.

RESULTS

Study of MTS from wild-type and MTS-defective strain (H.19.3.4). The data in Table 1 show assays of MTS from the mutant and wild-type strains. It can be seen that in the mutant the apparent K_m for methionine is at least 200-fold higher than in the wild type. The K_m for ATP is of the same order of magnitude in both strains, whereas the K_m for RNA is five times lower in the mutant than in the wild type. It must be emphasized that the K_m determinations are less accurate for the mutant than for the wild type due to the low affinity for methionine of the MTS extracted from strain H.19.3.4. Nevertheless, the greatest modification observed is in the K_m for methionine, which agrees with our previous findings about regulation and $tRNA^{met}$ charging in this strain.

TABLE 1. *Activities and K_m values of the MTS extracted from wild-type and mutant strains*

Strain[a]	MTS activity[b]	K_m[c]		
		L-Methionine (mM)	ATP (mM)	tRNA (mg/ml)
4094-B	1.4	0.005	5	3
H.19.3.4	0.042	1	10	0.6

[a] Complete genotypes are given in the text.

[b] Expressed as nanomoles/10 min per milligram of protein measured at 0.1 mM L-methionine, which is saturating for the wild-type enzyme.

[c] The range of concentrations used is given in the text. These determinations were performed on purified preparations of MTS to eliminate tRNA's. The preparation technique will be published elsewhere (Y. Surdin-Kerjan, unpublished data).

Study of SAM-mediated repression in the MTS-modified strain. In a previous report we had shown that SAM per se exerted a potent repressive effect on the synthesis of met group I enzymes in a wild-type strain (2). Moreover, we had studied ethionine-resistant strains in which repression by exogenous methionine was suppressed while SAM-mediated repression remained as efficient as in a wild-type strain (3). It was then of interest to know whether, in the MTS-deficient strain, met group I enzyme synthesis would be repressed and $tRNA^{met}$ would be fully charged in vivo after growth in a SAM-supplemented medium. The results (Table 2) clearly indicate that 0.15 mM L-SAM promotes the same percentage of repression of both homocysteine synthetase and ATP sulfurylase as the one found in a wild-type strain (2). In addition, one can see that, whereas the repression exerted by 4 mM exogenous DL-methionine is accompanied by a large amount of met-$tRNA^{met}$ present in vivo, the repression promoted by 0.15 mM SAM corresponds to a small amount of met-$tRNA^{met}$ present in vivo, this low amount being comparable to that found after growth in non-repressive conditions (0.1 and 0.2 mM exogenous DL-methionine).

Study of methionine and SAM pools in the MTS-modified mutant. In a wild-type strain, we have shown that SAM-mediated repression is not due to an in vivo reconversion of SAM into methionine (2). Conversely, it was difficult to eliminate the possibility that methionine-mediated repression could be due to its in vivo activation into SAM, since it is well known that growth of *S. cerevisiae* in the presence of high exogenous concentrations of methionine leads to high endogenous accumulation of both methionine and SAM (2, 22). From the study of methionine and SAM pools in the MTS-modified strain (Table 3), it appears that growth in SAM-supplemented media, leads, as expected, to a large accumulation of endogenous SAM but does not modify the endogenous methionine pool. Moreover, it can be seen that after growth in the presence of 4 mM DL-methionine the methionine endogenous pool is greatly enhanced. Under these conditions, the SAM endogenous pool is only 2 μmol/g of dry cells. As we have reported previously (2),

TABLE 2. *Comparison between SAM and methionine effects on the level of two met group I enzymes and on the degree of in vivo tRNA^{met} charging in the MTS-modified strain*[a]

Addition to minimal medium (mM)	Homocysteine synthetase		ATP sulfurylase		Charged tRNA^{met} in vivo (%)
	Sp act	Repression (%)	Sp act	Repression (%)	
DL-Methionine, 0.1[b]	235		140		21[c]
DL-Methionine, 0.2	200	15	100	28.5	19[c]
DL-Methionine, 4	49	79	40	71.5	92[c]
DL-Methionine, 0.1, + L-SAM, 0.15	50	79	34	75.5	21

[a] Growth was at 28 C.

[b] Some methionine addition to the culture medium is required to permit growth of strain H.19.3.4. DL-Methionine (0.1 mM) does not lead to any repression on methionine biosynthetic enzymes even in a wild-type strain (4).

[c] The differences between these values and the ones reported previously (4) are a consequence of different methods of tRNA extraction.

TABLE 3. *Methionine and SAM pools*[a] *in a mutant carrying a modified MTS after growth*[b] *under different conditions*

Addition to minimal medium (mM)	Methionine pools	SAM pools
DL-Methionine, 0.1[c]	2.6	0.75
DL-Methionine, 0.2	2.7	0.9
DL-Methionine, 4	54	2
DL-Methionine, 0.1, + L-SAM, 0.15	2	10

[a] Pools are expressed as micromoles per gram (dry weight).

[b] Growth was at 28 C.

[c] In a wild-type strain grown for several generations in a medium supplemented with 0.1 mM DL-methionine, methionine and SAM pools are similar to those found in the absence of any addition; both methionine and SAM pools are of the order of magnitude of 1 μmol/g (dry weight).

such an endogenous SAM pool built from exogenous SAM in a wild-type strain is unable to promote any repression of enzyme synthesis. Still, it could be questionable whether such a pool built from exogenous methionine would repress enzyme synthesis. This would imply a different value for repression mediated by biosynthesized SAM and SAM taken from the medium. Although in strain H.19.3.4 the SAM pool is low after growth in the presence of 4 mM DL-methionine as compared with that in a wild-type strain grown under the same conditions (2), it is difficult to draw a firm conclusion. In any case, this low pool seems to be in the favor of the independence of methionine and SAM actions. This conclusion is strengthened by results obtained in the study of kinetics of derepression of enzyme synthesis, where it is shown that the repressor synthesized from exogenous methionine cannot be in equilibrium with the SAM endogenous pool (Y. Surdin-Kerjan and H. de Robichon-Szulmajster, J. Bacteriol., submitted for publication). The low accumulation of endogenous SAM from exogenous methionine in the MTS mutant strain can be compared with the same phenomenon reported for mutants exhibiting a modified pattern of methionine-mediated repression (3). This non-accumulation of SAM cannot be attributed to some defect in methionine adenosyl transferase since its activity, at least in vitro, was found to be unimpaired in strain H.19.3.4 as well as in the regulatory mutants.

Up to this point, it was questionable whether all these features observed in strain H.19.3.4 were due to the same mutation. Therefore, we decided to examine the repartition of these properties among segregants of a diploid resulting from a cross between strain H.19.3.4 and a wild-type strain (diploid CC196). A thorough genetic study of the segregation of the various properties reported above was not undertaken because the only property we could detect easily was the thermosensitivity. For the other properties such as the endogenous methionine and SAM pools, the regulatory patterns exerted by methionine or SAM, and the corresponding degree of in vivo tRNA^{met} charging, extracts of each segregant after growth under various conditions would be necessary. In fact, we tried to isolate a segregant bearing only a modified MTS to determine whether the regulatory pattern of strain H.19.3.4 could be attributed only to the MTS modification. We first examined, in a few tetrads, the deficiency in MTS activity and the segregation of the thermosensitive character. In tetrad CC196-6

we found a thermoresistant spore carrying a mutant-type MTS. Therefore, we undertook a detailed study of this tetrad. It should be emphasized that all auxotrophic characters were present in the parental strains segregate as expected, except the methionine requirement, already reported as difficult to score due to its variable expression (19); it will be studied with the other properties.

Study of the thermosensitive character in each segregant from tetrad CC196-6. Results from the study of thermosensitive characters are reported in Table 4. It can be seen first that at 28 C only one segregant, CC196-6B, is unable to grow in the absence of exogenously added methionine, which is one of the characteristics of the mutant H.19.3.4. If we look now at the results of growth at 38 C, it can be seen that there are two thermosensitive spores: CC196-6B and CC196-6C. However, it must be remarked that strain CC196-6C can grow at 38 C in complex YPGA medium. This explains the fact that in some tetrads we found that the segregation of thermosensitivity on YPGA solid medium appears to be two thermoresistant, one thermosensitive, and one leaky strain. This was the case for the tetrad presented here.

Study of MTS activity in each segregant of tetrad CC196-6C. The K_m for methionine has been determined for the MTS extracted from the four spores of the tetrad CC196-6. It has been found that the MTS extracted from spores 6B and 6D has the same apparent affinity for methionine as the enzyme from strain H.19.3.4 (K_m = 0.8 and 0.5 mM for 6B and 6D, respectively), whereas the enzyme extracted from spores 6A and 6C exhibits the same K_m for methionine as the wild-type strain (K_m = 0.005 mM). So, it can be seen that in tetrad CC196-6 the MTS lesion has been dissociated from the thermosensitivity, since spore CC196-6D is thermoresistant and carries a modified MTS.

To determine whether the thermosensitivity could be related to another modification of the MTS, we tested whether the enzyme present in the strain that only retained the thermosensitive character (CC196-6C) is thermolabile. We found that at 42 C the half-life of the MTS extracted from CC196-6C is identical to the one of the MTS extracted from the wild-type strain belonging to the same tetrad (CC196-6A). It seems then unlikely that the thermosensitivity of spore CC196-6C could be the result of a modification of the MTS in this strain.

Regulatory pattern and in vivo charging of tRNA in tetrad CC196-6. If we consider the two segregants (CC196-6A, thermoresistant, and CC196-6C, thermosensitive) that carry a wild-type MTS, the results (Table 5) show that the synthesis of met group I enzymes is already repressed by 0.2 mM DL-methionine, which corresponds to a wild-type repression pattern. As

TABLE 4. *Mean generation time of each spore from tetrad CC196-6 at 28 and 38 C under various growth conditions*

Growth temp (C)	Addition to minimal medium (mM)	Mean generation time (h)			
		CC196-6A	CC196-6B	CC196-6C	CC196-6D
28	None	4	∞	4	3
	DL-Methionine, 0.1	4.15	6.30	4	3
	DL-Methionine, 0.2	4.15	6.30	4	3
	DL-Methionine, 4	4.30	5	5	3
	DL-Methionine, 0.1, + L-SAM, 0.15	—[a]	6.30	—	3.30
	L-SAM, 0.15	—	∞	—	3
	YPGA[b]	2.30	3	2.30	2.30
38	None	4.15	∞	∞	3.30
	DL-Methionine, 0.1	4.15	∞	∞	3.30
	DL-Methionine, 0.2	4.15	∞	∞	3
	DL-Methionine, 4	5	∞	∞	3
	DL-Methionine, 0.1, + SAM, 0.15	—	∞	—	—
	L-SAM, 0.15	—	∞	—	—
	YPGA	2.30	∞	4.30[c]	2.30

[a] —, Not done.

[b] YPGA, yeast extract; peptone (Difco), 5 g/liter; glucose, 30 g/liter; adenine, 40 mg/liter.

[c] After a lag period of about 8 h.

TABLE 5. *Level of two met group I enzymes and of tRNA^{met} in vivo charging in each spore of tetrad CC196-6 under various growth conditions*[a]

Spore no.	Addition to minimal medium (mM)	Homocysteine synthetase (% repression)	ATP sulfurylase (% repression)	Charged tRNA^{met} in vivo (%)
CC196-6A	None			86
	DL-Methionine, 0.1	0	0	100
	DL-Methionine, 0.2	69	87	85
	DL-Methionine, 4	77	97	100
CC196-6B	DL-Methionine, 0.1			30
	DL-Methionine, 0.2	0	20	30
	DL-Methionine, 4	77	80	100
	DL-Methionine, 0.1, + L-SAM, 0.15	90	70	10
CC196-6C	None			86
	DL-Methionine, 0.1	0	0	88
	DL-Methionine, 0.2	66	80	94
	DL-Methionine, 4	82	95	86
CC196-6D	None			28
	DL-Methionine, 0.1	0	0	26
	DL-Methionine, 0.2	0	0	20
	DL-Methionine, 4	75	100	94
	L-SAM	63	85	24

[a] Growth was at 28 C.

expected, the level of met-tRNA^{met} in vivo is high in these two strains, whatever the culture conditions. This has already been reported in a wild-type strain. It had been taken as an argument for the participation of only a minor species of tRNA^{met} in regulation (4). If we consider now the two other segregants (CC196-6B, thermosensitive, and CC196-6D, thermoresistant) that bear a modified MTS, results (Table 5) show that 0.2 mM DL-methionine is unable to promote a noticeable repression of synthesis of met group I enzymes, which is paralleled by the presence of a small amount of in vivo met-tRNA^{met}. As expected, growth in the presence of 4 mM DL-methionine promotes both repression of met group I enzymes and reacylation of tRNA^{met} in vivo. In addition, 0.15 mM L-SAM, exogenously added, leads also to a potent repression of met group I enzymes but is not accompanied by a concomitant recharging of tRNA^{met} in vivo. It seems then that in tetrad CC196-6 the presence of the modified MTS is accompanied by a modified repression pattern by exogenous methionine. To be sure that these two characters are linked, we crossed spore 6D with a wild-type strain and tested the segregants. It was found, without exception, that in 25 tetrads (100 spores) the MTS modification segregates as a monogenic character and that the modified regulatory pattern segregates with the MTS modification. These results are in favor of a role of MTS or a product of its activity in methionine-mediated repression.

Study of endogenous methionine and SAM pools of tetrad CC196-6. It can be seen in Table 6 that in all four strains the endogenous methionine pool is greatly enhanced after growth in the presence of 4 mM DL-methionine as compared with the basal level (obtained after growth in minimal medium). This basal level is quite the same for each segregant and similar to the one found in a wild-type strain, with the exception of CC196-6B. In this segregant, as in the original mutant strain H.19.3.4, we observed a slightly elevated basal level of methionine. As far as the SAM pools size are considered, it can be seen that in the two MTS-modified mutants (CC196-6B and CC196-6D) growth in the presence of 4 mM DL-methionine only leads to a two- to threefold increase of the endogenous amount of SAM as compared with the one obtained after growth in minimal medium. It could be expected then that in the two segregants showing a wild-type MTS activity the enhancement of the endogenous SAM pool after growth in the presence of a high methionine concentration would be comparable to that observed in a wild-type strain. This was found to be the case in the

TABLE 6. *Methionine and SAM pools in segregants of tetrad CC196-6*

Spore no.	Addition to minimal medium (mM)	Methionine pool[a]	SAM pool[a]
CC196-6A	None	0.75	1.33
	DL-Methionine, 4	194	4.3
CC196-6B	DL-Methionine, 0.1	2.2	1.6
	DL-Methionine, 4	184	3.6
CC196-6C	None	0.5	1.25
	DL-Methionine, 4	198	10.25
CC196-6D	None	1	1.3
	DL-Methionine, 4	86	3.7

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

strain CC196-6C but not in the strain CC196-6A. Strain CC196-6C displays a 10-fold increase in its endogenous SAM pool, whereas in CC196-6A only a 3.5-fold increase was obtained. We have no explanation for the behavior of this last strain, but it could be the result of an additional genetic lesion present in the parental strain.

DISCUSSION

The study of strain H.19.3.4 presented here shows that this mutant carried an MTS exhibiting a 200-fold increase in its K_m for methionine as compared with the wild-type enzyme. This results in a small amount of in vivo met-tRNA^{met} and a modified pattern of repressibility of met group I enzymes, as reported previously (4). This drastically reduced affinity of the mutant enzyme probably contributes to the apparent methionine requirement of strain H.19.3.4 despite normal methionine biosynthesis. Strain H.19.3.4 was also found to be thermosensitive, but it was shown previously that neither the level of in vivo tRNA^{met} charging nor the modified repressibility of met group I enzymes synthesis appears to be different at the permissive (28 C) and at a semipermissive (34 C) temperature (4).

From their genetic study of strain H.19.3.4, McLaughlin and Hartwell (19) assumed that the mutation resulting in the defective MTS and the one leading to thermosensitivity are probably identical. It seems that such a clear-cut statement cannot be made in view of the results reported here, which at least imply that the mutation leading to the thermosensitivity is not in the structural gene coding for MTS and does not lead to an additional modification of the MTS. In fact, our results are in agreement with some observations reported by McLaughlin and Hartwell, such as the fact that among four thermoresistant revertants of strain H.19.3.4 tested for MTS activity two are still devoid of this activity. Considering the origin of the MTS mutant (heavy nitrosoguanidine mutagenesis) it is not surprising that strain H.19.3.4 is a multiple mutant. The thermosensitivity and the MTS defect being dissociated, it appears that the modified pattern of repressibility by exogenous methionine remains associated with the latter character. It has been verified that in a cross between CC196-6D and a wild-type strain, the MTS defect and the modified pattern of repressibility by methionine segregate together, showing good evidence that these two features are the consequence of the same mutation.

The regulation of met group I enzymes synthesis by methionine and the amount of in vivo met-tRNA^{met} appear to be similar in strains H.19.3.4 and CC196-6D, i.e., growth in the presence of a low methionine concentration corresponds to a small in vivo amount of met-tRNA^{met} and an absence of repressibility of met group I enzymes synthesis, whereas growth in the presence of a high methionine concentration corresponds to the recovery of repression of met group I enzymes synthesis and to a large in vivo amount of met-tRNA^{met}. This last feature arises probably from the increase of the endogenous methionine pool size, which permits saturating the MTS in vivo; in addition, and as noted above, the endogenous SAM pool remains quite low compared with that measured in cells of a wild-type strain grown in minimal medium (2).

We have previously reported that, besides methionine, SAM per se is able to promote complete repression of met group I enzymes synthesis in a wild-type strain (2). Results reported in this paper show that the same SAM concentration leading to repression of met group I enzymes in a wild-type strain also promotes a similar level of repression of these enzymes in strains H.19.3.4 and CC196-6D. In addition, it appears that SAM-mediated repression does not lead to any increase of either the pool size of free methionine or the amount of met-tRNA^{met} present in vivo, whereas the endogenous SAM pool is increased by about 10-fold. Thus, H.19.3.4 and CC196-6D are typical strains which bring additional evidence that exogenous methionine and exogenous SAM exert independently their repressive effects. Results to be published (Surdin-Kerjan and de Robichon-Szulmajster, J. Bacteriol., submitted for publication) suggest that methionine (probably via methionyl-tRNA) might act at the

transcriptional level and SAM acts at the translational level.

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