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Elements Involved in *S*-Adenosylmethionine-Mediated Regulation of the *Saccharomyces cerevisiae MET25* Gene

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In *Saccharomyces cerevisiae*, the *MET25* gene encodes *O*-acetylhomoserine sulfhydrylase. Synthesis of this enzyme is repressed by the presence of *S*-adenosylmethionine (AdoMet) in the growth medium. We identified *cis* elements required for *MET25* expression by analyzing small deletions in the *MET25* promoter region. The results revealed a regulatory region, acting as an upstream activation site, that activated transcription of *MET25* in the absence of methionine or AdoMet. We found that, for the most part, repression of *MET25* expression was due to a lack of activation at this site, reinforced by an independent repression mechanism. The activation region contained a repeated dyad sequence that is also found in the promoter regions of other unlinked but coordinately regulated genes (*MET3*, *MET2*, and *SAM2*). We show that the presence of the two dyads is necessary for maximal gene expression. Moreover, we demonstrate that in addition to this transcriptional regulation, a posttranscriptional regulation, probably targeted at the 5′ region of mRNA, is involved in *MET25* expression.

Synthesis of the enzymes of methionine metabolism in *Saccharomyces cerevisiae* is repressed during growth in the presence of methionine. This amino acid is transformed rapidly into *S*-adenosylmethionine (AdoMet), and we have previously shown that externally added AdoMet promotes repression of synthesis of the same enzymes as does methionine (5). Further studies have shown that intracellular AdoMet is the true effector of this negative regulation; therefore, it can be considered the end product of the methionine pathway (5, 6, 27).

As shown by Cantoni (2), AdoMet is a ubiquitous metabolite involved in almost as many reactions as ATP. The precise balance of the internal pool of AdoMet is therefore probably crucial for the cell, and this balance is evidently due to the regulation of AdoMet biosynthesis. An interesting feature of AdoMet metabolism is that *S. cerevisiae* is able to resynthesize methionine from metabolites resulting from AdoMet utilization. Two pathways are known to synthesize methionione from AdoMet in yeast cells; the first leads from *S*-adenosylhomocysteine (resulting from the demethylation of AdoMet) to homocysteine and then to methionine, and the second leads directly to methionine from 5′-methylthioadenosine (produced from AdoMet mainly in the biosynthesis of polyamines). As emphasized by Tabor et al. (26), in *Escherichia coli* about 90% of intracellular AdoMet is involved in methylation. If the same figure applies in the case of *S. cerevisiae*, the cycle back to methionine from AdoMet would go mainly by the intermediary of *S*-adenosylhomocysteine and homocysteine. These salvage pathways are of interest because they would allow the cell to bypass the sulfate assimilation pathway, which requires a high expenditure of energy. This would require that the de novo synthesis of methionine from its constituents be tightly regulated. Homocysteine is the branch point of the biosynthesis of methionine and AdoMet from *O*-acetylhomoserine on the one hand and of the main salvage pathway to methionine from AdoMet on the other hand. The de novo formation of homocysteine from *O*-acetylhomoserine and sulfur is a reaction catalyzed by *O*-acetylhomoserine sulfhydrylase, encoded by the *MET25* gene. Thus, regulation of the synthesis of this enzyme is important with respect to energy when the cell uses the salvage pathways.

With every yeast gene studied so far, expression above the basal level requires an activator that binds to an upstream activation site (UAS) (for reviews, see references 1 and 8). Some yeast genes have, in addition to the activation site, an operator site which mediates negative control. The best-characterized repressor in yeast cells is the MATα2 protein, which has been shown to bind directly to a specific operator sequence (14). However, the mechanism of repression, especially in metabolic pathways, is far from being as well understood as gene activation (8).

We decided to study the molecular mechanism underlying the negative regulation acting on the expression of *MET* genes in *S. cerevisiae*. We chose *MET25*, which catalyzes the synthesis of homocysteine from *O*-acetylhomoserine, because, for the reasons cited above, we suspected that regulation of its expression would be more complex than for the other *MET* genes. Therefore, a further type of regulation, superimposed on that acting on the other *MET* genes, could perhaps be identified.

We report here a functional analysis of the *MET25* promoter and new information on the regulation of synthesis of the enzyme encoded by *MET25*.

MATERIALS AND METHODS

Strains. The strains used are listed in Table 1. *E. coli* HB101 and JM103 were used as hosts for plasmid maintenance and recovery.

Media. Media for growth of *S. cerevisiae* and *E. coli* were as described by Sherman et al. (23) and Maniatis et al. (17).

Recombinant DNA methods. Plasmid purification was performed as described by Ish-Horowicz and Burke (9). Northern (RNA) blotting with total cellular RNA was done as described by Thomas (29). Probes were made radioactive by random-priming DNA labeling as described by the supplier.

Transformation. *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. (10).

Vectors and plasmid constructions. For sequencing, we

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TABLE 1. *S. cerevisiae* strains used

Strain	Genotype	Source or reference
CC359-OL2	*ura3 leu2 his3*	H. Cherest
CC435-6C	*ura3 leu2 his3 sam1::URA3 sam2::URA3*	27
CC375-3C	*ura3 lys2 lys5*	H. Cherest
C109	*ura3 lys2 lys5 met25::URA3*	This work
C114	*ura3 his3 MET25-lacZ* fusion inserted at the *LEU2* locus	This work

used the bacteriophage vector M13mp19. Plasmid pM25-24 was plasmid pUC19 into which we had inserted the *Bam*HI-*Xba*I fragment of pM25-5 containing the whole 5′ region of the *MET25* gene (22). Plasmid pLM1 was constructed as follows (Fig. 1). Plasmid pUC9 was digested by endonucleases *Bam*HI and *Sal*I and treated with the Klenow enzyme to blunt the ends. The *Xho*I-*Sal*I fragment of plasmid YEP13 containing the *LEU2* gene was also blunt ended and ligated into the vector, which destroyed the *Bam*HI site. The resulting plasmid was then cut by endonuclease *Cla*I, treated with the Klenow enzyme, and ligated to the *Bam*HI-*Hpa*I site of pM25-5 (containing the whole *MET25* gene), also treated with the Klenow enzyme; this re-formed a *Bam*HI site. This plasmid contains the *LEU2* gene interrupted by the *MET25* gene. To make constructs with deletions in the *MET25* gene, the *Bam*HI-*Xba*I wild-type fragment could be replaced by the same fragment bearing a deletion (see below). For regulation studies, the DNA fragment containing the whole 5′ region and the first 17 codons of the *MET25* gene (pM25-5; 12) was fused in frame with a *lacZ* gene that lacks the first 8 codons (3).

Deletions. To make 5′ deletions, plasmid pM25-24 was cut at the *Bam*HI site, and the linearized plasmids were treated with exonuclease *Bal*31 (0.1 U/μg of DNA, 25°C, 1, 3, and 6 min). The DNA was then treated with the Klenow fragment to blunt ends, ligated to *Eco*RI linkers (GGAATTCC), and cut by *Eco*RI and *Xba*I. The *Eco*RI-*Xba*I fragments of different sizes were isolated by agarose gel electrophoresis and inserted into bacteriophage M13mp19 DNA cut by *Eco*RI and *Xba*I. The 3′ deletions were prepared similarly, the starting vector being cut at the *Xba*I site and the deleted fragments inserted into bacteriophage M13mp19 DNA cut by *Eco*RI and *Bam*HI. Sizes of the different deletions were estimated by endonuclease digestion and agarose gel analysis, and all of the fragments used in deletion constructions were sequenced.

DNA sequencing. Nucleotide sequences of the fragments containing deletions recloned in phage M13mp19 DNA were determined by the dideoxy-chain termination method (21) after isolation of single-stranded DNA (18).

Oligonucleotide replacement. Oligonucleotides containing the two 8-base-pair (bp) sequences that are repeated in the UAS region of *MET25* were synthesized. The sequences of the two synthetic duplexes (oli_A and oli_B) are shown in Fig. 7. The oligonucleotides were designed so that each end of the synthetic duplex formed an *Eco*RI site and also so that an *Xho*I site was at one end of the oli_A duplex and a *Sma*I site was at one end of the oli_B duplex; thus, the presence of these oligonucleotides could be ascertained. After synthesis, the oligonucleotides were annealed and ligated into the *Eco*RI site of the deletion mutant. Every construct was sequenced.

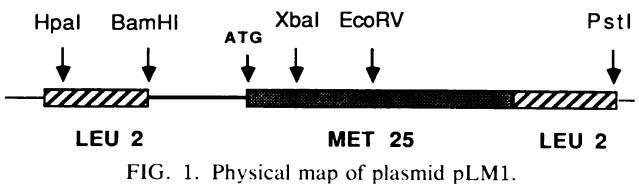


FIG. 1. Physical map of plasmid pLM1.

Enzymatic assay. Cells from 10-ml cultures in the exponential phase of growth were centrifuged, washed once with extraction buffer, and suspended in 0.2 ml of the same ice-cold buffer. About 0.2 ml of glass beads (0.45-mm diameter) was added, and the cells were disrupted by agitation with a vortex mixer at maximum speed (10-s agitation, 1-min cooling on ice), repeated 10 times. After 0.5 ml of the same buffer was added, cell debris and glass beads were eliminated by centrifugation at 0°C. For *O*-acetylhomoserine sulfhydrylase, the extraction buffer was 100 mM Tris hydrochloride (pH 8); enzyme activity was assayed as described by Wiebers and Garner (30), the homocysteine formed being estimated by the method of Kredich and Tomkins (13). For β-galactosidase, the extracts were made in Z buffer, and enzyme activity was assayed as described by Miller (19).

RESULTS

Strategy for promoter analysis. Sequenced *Bam*HI-*Eco*RI and *Eco*RI-*Xba*I fragments (see Materials and Methods) were ligated to obtain internal deletions of known sizes. The *Bam*HI-*Xba*I fragment of *MET25* of plasmid pLM1 (Fig. 1) was removed and replaced by a *Bam*HI-*Xba*I fragment bearing a deletion. The resulting plasmid was cut by *Pst*I and *Hpa*I and used for chromosomal *LEU2* gene disruption (20) in strain C109, in which the chromosomal *MET25* gene had been inactivated by insertion of the *URA3* gene. By selecting transformants phenotypically Met^+ and Leu^-, we obtained strains in which a defined region of the *MET25* promoter was removed and replaced by an *Eco*RI site. The disruption of *LEU2* by *MET25* was verified by Southern blotting in several of the strains bearing the deletions (results not shown).

An upstream region regulates transcription from the *MET25* promoter. (i) Enzymatic analysis. For each deletion, at least two strains were tested for *O*-acetylhomoserine sulfhydrylase activity (Fig. 2). With respect to enzyme activities after growth in minimal medium, a regulatory region located about 300 bp upstream of the ATG was localized by the overlapping deletions ΔF, ΔG, and ΔH to a 108-bp region (between positions −253 and −362), the flanking deletions ΔE and ΔI expressing about 50% of the wild-type enzyme activity. We tried to narrow this regulatory region to less than 108 bp by constructing deletions ΔF1, ΔF2, ΔF3, and ΔF4 (Fig. 3). *O*-Acetylhomoserine sulfhydrylase activities found in the strains with these deletions (except for ΔF3) were somewhat higher than the activity found in the strain with the ΔF deletion.

With respect to *O*-acetylhomoserine sulfhydrylase activity after growth in the presence of methionine (repressing conditions), we did not identify a region which, once deleted, would lead to a complete loss of repression (Fig. 2). Nevertheless, deletions ΔA, ΔB, ΔC, and ΔD showed three to eight times more enzyme activity than did the control grown in the same conditions. These deletions covered a 105-bp region (positions −270 to −165) containing the sequence 5′-GAAA ACTGTGG-3′, which has also been found in the 5′ regions of the coregulated genes *SAM1*, *SAM2*, *MET2*, and *MET3* (27; Fig. 4). This sequence is probably involved in repression, although not by a simple mechanism because its deletion, even in ΔC, which lacked only 35 bp of the *MET25*

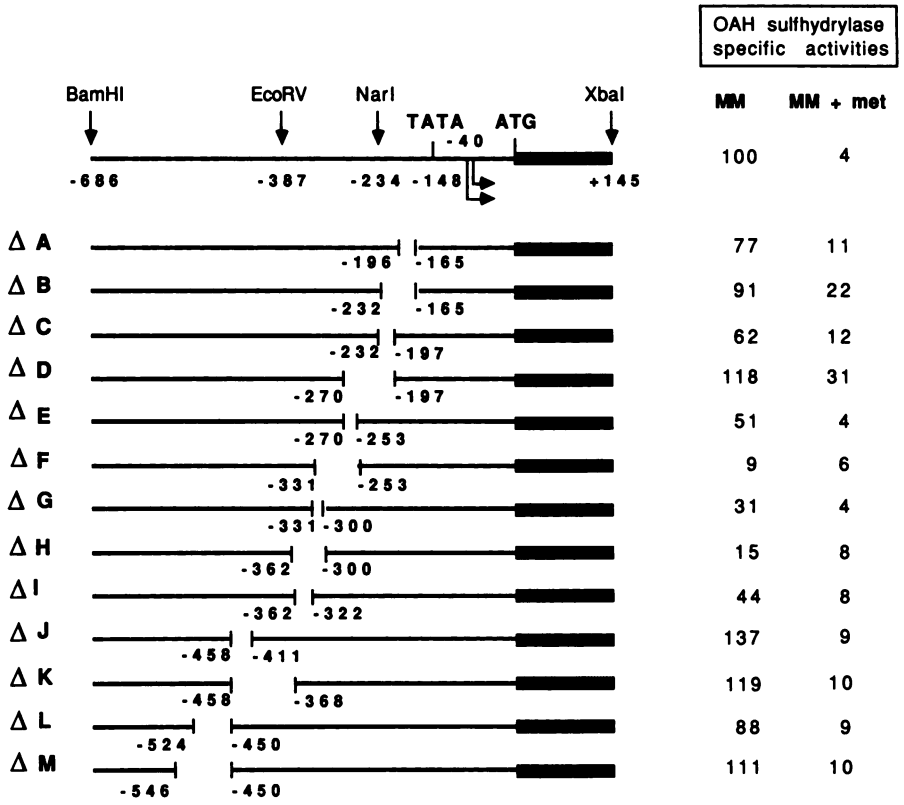


FIG. 2. *MET25* promoter and its derivatives with deletions. The restriction map shows 831 bp covering the promoter region of *MET25*. Transcription starts are indicated by arrows; the TATA box and ATG are marked. The deletions were made as described in the text. For each deletion-bearing strain, specific activity of the *MET25* enzyme is reported and expressed as a percentage of the activity encoded by the *MET25* gene bearing the wild-type promoter inserted at the same locus (see Materials and Methods). The mean value of at least two assays (four assays in some cases) was calculated. For growth in minimal medium, the results are within 10% of the given value; after growth in the presence of methionine, they could differ by up to 15% of the given value. Abbreviations: OAS sulfhydrylase, *O*-acetylhomoserine sulfhydrylase; MM, minimal medium; MM + met, minimal medium containing 2 mM DL-methionine.

promoter region, resulted only in a modification of the level of repression (75 to 80% versus 95% in the control). In strains with deletions ΔF and ΔF1 to ΔF4 (Fig. 3), *O*-acetylhomoserine activity was lower after growth in methionine than after growth in minimal medium.

(ii) Northern blot analysis. After growth in minimal medium of strains bearing different deletions, total RNA was extracted and used for Northern blotting, with a radioactive *MET25* fragment used as a probe. The results (Fig. 5) were in accord with results of the enzymatic analysis. The Northern filters were also probed with a radioactive fragment of the actin gene; in this case, no significant difference was found among the different RNAs. The same strains grown in the presence of methionine showed repression of the level of steady-state *MET25* mRNA and less repression for deletions ΔB, ΔC, and ΔD (data not shown).

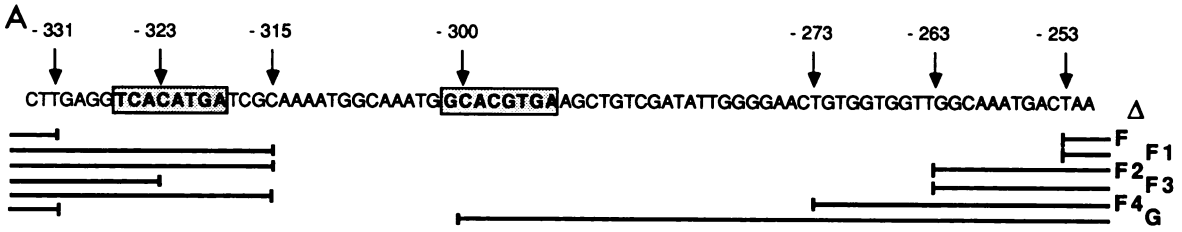
The *MET25* regulatory region contains short sequences with dyad symmetry related to sequences of *MET2*, *MET3*, and *SAM2*. We analyzed the region deleted in ΔF for sequences that are also present in the promoter regions of genes *MET2*, *MET3*, *SAM1*, and *SAM2*, which have the same regulatory pattern as does *MET25* (4, 15, 27, 28). We found a short sequence, 5′-TCACGTGA-3′, that occurred twice in the regulatory region of *MET25* as defined by the ΔF deletion (boxed in Fig. 3). This 8-bp sequence was found in the 5′ regions of three of the related genes cited above but not in *SAM1* (Fig. 6). Analysis of the results of Fig. 2 and 3 in relation to these sequences showed that deletions ΔF and ΔF3, lacking both sequences, had the lowest activity, 10% of the control level. Deletions ΔF1, ΔF2, and ΔF4, lacking the second regulatory sequence, had about 15% of the control activity. The ΔG deletion, which lacked the first regulatory sequence, had 30% of the control level of activity.

ΔH, which had low activity (15% of the control level), differed from ΔG, which was more active, only by the deletion of stretches of thymine residues that were found upstream of the ΔF deletion. This fact suggests that these thymine residues may play a role in transcription of the gene, as has been shown for the *HIS3* gene (24). Accordingly, in ΔI the same stretches of thymine residues, as well as 5 of 8 bp of the first regulatory sequence, were deleted, resulting in 44% of the control enzyme activity level.

ΔE, deleted from positions −253 to −270, had only 50% of the control level of activity. We have no explanation for the low activity of ΔE, but it could have resulted from either a spacing effect or the presence of a flanking sequence element. Nevertheless, ΔE is a short deletion, and we have not found in this region any sequence repeated in the regions flanking the dyads in the other coregulated genes.

Sequences with dyad symmetry function as UASs. To test whether the sequences determined above acted effectively as UASs, we synthesized two duplexes (oli_A and oli_B) as described in Materials and Methods. These two synthetic duplexes were inserted into ΔF, ΔF4, or ΔG, and *O*-acetyl-

A



B

Δ	Specific Activities	
	Minimal Medium	Minimal Medium + Met 2 mM
T	100	4
ΔF	9	6
ΔF1	14	7
ΔF2	16	5
ΔF3	10	4
ΔF4	14	7
ΔG	31	4

FIG. 3. Analysis of the ΔF deletion. (A) Sequence of the region deleted in construct ΔF, showing additional deletions constructed in this region. Boxes show the short dyads discussed in Results. (B) *O*-Acetylhomoserine sulfhydrylase activities expressed as a percentage of the activity encoded by the *MET25* gene bearing the wild-type promoter (T) shown in Fig. 2.

homoserine activity was tested in the strains bearing these new mutant *MET25* genes (Fig. 7). The oli_A duplex inserted in ΔF was equivalent to ΔF4 with respect to the regulatory sequences; activity increased from 9 to 14% of the control level. The same duplex, inserted in ΔF4 in one or the other direction, increased activity to 26% of the control level. The oli_B duplex inserted in ΔF or ΔF4 resulted in about 45% of the control level of activity. If the oli_B duplex was inserted in the ΔG deletion (this construction results in the presence of two copies of oli_B), recovery of *O*-acetylhomoserine sulfhydrylase activity was 100%. We can conclude that when oli_B was present, regardless of the presence of oli_A, *O*-acetylhomoserine sulfhydrylase activity was recovered at 45% of the control level, which is near the maximal activity that could be reached if we consider the 50% activity of the ΔE deletion. When two copies of oli_A were present, activity reached only 25% of the control level. When two copies of oli_B were present, 100% enzyme activity was reached. In all cases, *O*-acetylhomoserine sulfhydrylase synthesis was sensitive to repression.

Evidence for posttranscriptional control of *MET25* expression. We have shown previously, using strain CC435-6C doubly disrupted for the *SAM1* and *SAM2* genes and thus unable to transform methionine into AdoMet, that AdoMet, not methionine, is the external signal of repression of *O*-acetylhomoserine sulfhydrylase (27). In the study described here, we grew the parental strain CC359-OL2 and strain CC435-6C in different media, and the steady-state level of *MET25* mRNA was measured by Northern blotting (Fig. 8). For strain CC359-OL2, gene expression was completely repressed by growth in the presence of 2 mM DL-methionine or 0.2 mM AdoMet. With 0.05 or 0.1 mM AdoMet, the level of *MET25* mRNA was comparable to that after growth in minimal medium. For strain CC435-6C, expression of *MET25* was not decreased by addition of methionine to the growth medium, again providing evidence that AdoMet and

Gene		Sequence		Homology
MET25	-227	c a t **G A A A A C T G T G** t a a c	-211	10/11
MET2	-309	g c a **a A A A A** t **T G T G G** a t g	-293	9/11
SAM1	-282	c a g **G A A A A C T G T G G** t g g	-266	11/11
SAM2	-306	c t t **G A A A A C T G T G G** g t t	-280	11/11
MET3	-240	t t g **t A A A A C T G T G G** c t t	-256	10/11
Consensus		- - - **G A A A A C T G T G G** - - -		

FIG. 4. Putative repression-specific sequence found in coregulated genes of AdoMet metabolism. This sequence was found in the region deleted in ΔD and is homologous to sequences found in the 5'-flankings region of *MET2* (15), *SAM1* (28), *SAM2* (27), and *MET3* (4). Coordinates indicate positions with respect to the A of the presumptive start codon. Lowercase letters indicate bases different from those in the consensus sequence.

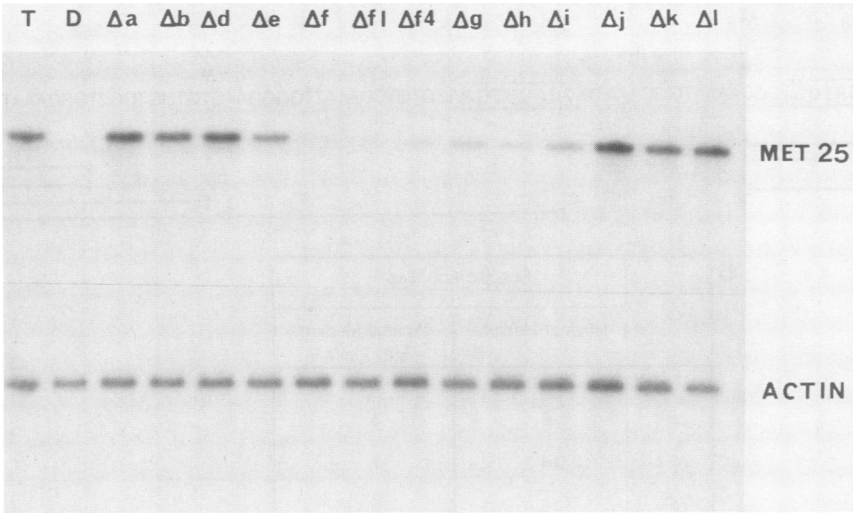


FIG. 5. Northern blot analysis of the *MET25*-specific mRNA in strains bearing the different deletions. Lanes: T, RNA extracted from the strain in which the wild-type gene was inserted in *LEU2* (see Materials and Methods); D, RNA extracted from strain C109, which was used for disruption of the *LEU2* gene by the different *MET25* alleles; Δa to Δl, RNA extracted from strains bearing the corresponding deletions. The RNAs were probed with a *MET25* probe and an actin probe.

not methionine is the external signal for regulation. Once again, the presence of 0.1 mM AdoMet in the medium did not promote repression of *MET25* mRNA synthesis, whereas the presence 0.2 mM AdoMet did.

The results of enzymatic assays (Table 2) showed that the level of *O*-acetylhomoserine sulfhydrylase paralleled the level of steady-state mRNA except when cells of both strains were grown in the presence of 0.1 mM AdoMet; in the latter case, the steady-state level of mRNA was maximal, whereas the specific enzyme activity was at its repressed level. This result indicates the existence of a posttranscriptional regulation of *O*-acetylhomoserine sulfhydrylase synthesis.

We made a gene fusion with *lacZ* as described in Materials and Methods. This fused gene was integrated near the chromosomal *LEU2* gene by conventional methods, yielding strain C114, and β-galactosidase activity was assayed in this strain after growth in different media. β-Galactosidase activity was not modified by growth in the presence of 0.05 mM AdoMet. When strain C114 was grown in the presence of 2 mM DL-methionine, 0.1 mM AdoMet, or 0.2 mM AdoMet, only 20% of the activity found in minimal medium was reached. These results parallel those found for *O*-acetylhomoserine dehydrogenase in strain CC359-OL2 (Table 2). The decrease in activity after growth in the presence of 0.1 mM AdoMet indicates that the observed posttranscriptional repression is most probably targeted at the 5′ region of *MET25* mRNA, although we cannot rule out a role of the first 17 amino acids of the *MET25*-encoded protein.

DISCUSSION

The experiments reported here reveal two important aspects of the regulation of expression of a *MET* gene in *S. cerevisiae*. First, we identified the presence on the *MET25* gene of a UAS that was not found by previous experiments using plasmids (22). This UAS contains a sequence of dyad symmetry that is repeated on the *MET25* promoter and also found repeated in the coregulated genes. Analysis of the effects of deletions ΔF, ΔF1, ΔF2, ΔF3, ΔF4, ΔG, and ΔH with regard to these conserved regulatory sequences showed that (i) the stretches of thymine residues situated upstream of the UAS are important for maximal *MET25* transcription,

Gene		Sequence		Homology
MET25	-303	t g **g C A C G T G A** a g c	-280	7/8
	-328	g g **T C A C a T G A** t c g	-315	7/8
MET2	-550	a a **T C A C G T G A** t a t	-537	8/8
	-356	t t **T C A C G T G A** t g c	-343	8/8
MET3	-381	g g **T C A C G T G A** c c a	-368	8/8
	-364	a g **T C A C G T G t** a a t	-351	7/8
SAM2	-326	a c **c C A C G T G A** c t a	-313	7/8
	-380	c t **T C A C a T G t** g a t	-367	6/8
Consensus		- - **T C A C G T G A** - - -		

FIG. 6. Sequence homologies found in the UAS region of *MET25* and in the 5′-flanking regions of coregulated genes of AdoMet metabolism. Sequences from the UAS region of *MET25* defined by the ΔF deletion are shown, along with homologous sequences from *MET2* (15), *MET3* (4), and *SAM2* (27). Coordinates indicate positions with respect to the A of the presumptive start codon. Lowercase letters indicate bases different from those in the consensus sequence.

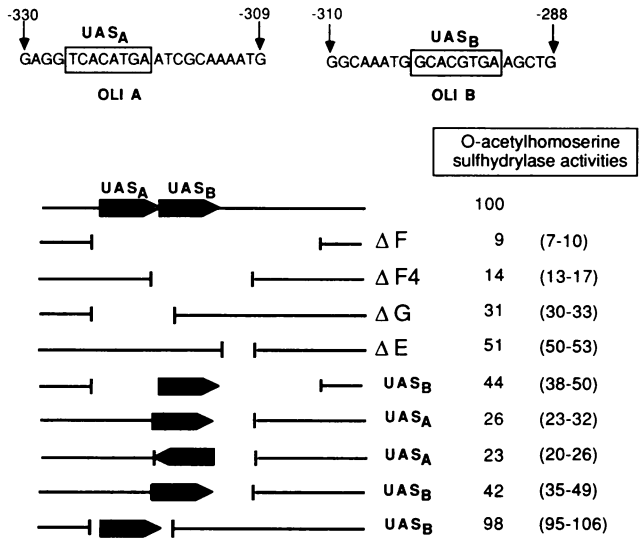


FIG. 7. Function of oli$_A$ and oli$_B$ as a UAS in *MET25* expression. The nucleotide sequences of the two duplexes, oli$_A$ and oli$_B$, containing the repeated sequences found in the UAS region are shown at the top. The synthetic duplexes were inserted into the *Eco*RI site of the ΔF or ΔF4 deletion as shown. Orientation of the insert was determined by DNA sequencing. *O*-Acetylhomoserine sulfhydrylase activity is presented as in Fig. 2. Ranges of values obtained in the different experiments are shown in parentheses.

(ii) the position of the dyads is probably important for maximal induction, and (iii) the presence of two dyads is necessary for maximal *MET25* expression.

This last point was further illustrated by the use of synthetic duplexes. The presence of one copy of oli$_B$ resulted in the highest attainable activity (about 43% of maximal activity and comparable to the activity of ΔE), whereas one copy of oli$_A$ resulted only in 26% of maximal activity. This result is certainly due to the fact that the core of this sequence is a perfect palindrome, CACGTG, whereas oli$_A$ contains the sequence CACATG. If two copies of oli$_B$ were

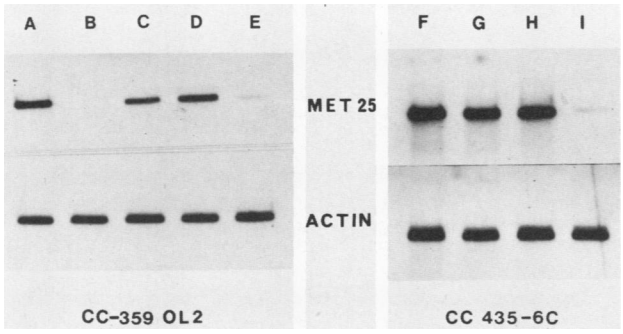


FIG. 8. Northern analysis of *MET25* mRNA extracted from strains CC359-OL2 (parental) and CC435-6C (*sam1*::*URA3* *sam2*::*URA3*) after growth in different conditions. Strain CC359-OL2 was grown in minimal medium supplemented with no addition (lane A), 2 mM DL-methionine (lane B), 0.05 mM AdoMet (lane C), 0.1 mM AdoMet (lane D), or 0.2 mM AdoMet (lane E). Strain CC435-6C was grown in minimal medium supplemented with 0.05 mM AdoMet (lane F), 0.05 mM AdoMet plus 2 mM DL-methionine (lane G), 0.1 mM AdoMet (lane H), or 0.2 mM AdoMet (lane I). The RNAs were probed with a *MET25* probe and an actin probe, showing that the RNA concentrations in all lanes were similar.

TABLE 2. *O*-Acetylhomoserine sulfhydrylase specific activities in strains grown in different conditions

Strain	Relevant genotype	Addition to minimal medium	Sp act[a]
CC359-OL2			220
		2 mM DL-methionine	20
		0.05 mM AdoMet	250
		0.1 mM AdoMet	48
		0.2 mM AdoMet	19
CC435-6C	*sam1*::*URA3* *sam2*::*URA3*	0.05 mM AdoMet	521
		0.05 mM AdoMet + 2 mM DL-methionine	412
		0.1 mM AdoMet	44
		0.2 mM AdoMet	32

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

present, 100% of the *O*-acetylhomoserine sulfhydrylase activity was reached.

We have analyzed deletions from the TATA box up to position −546 of the promoter region of *MET25*. This analysis has not provided evidence of a site which, once deleted, would give a constitutive level of transcription.

The second important result described here is the existence of posttranscriptional as well as transcriptional regulation. The existence of dual regulation and posttranscriptional regulation mediated by AdoMet has already been described. It was shown that untranslated *MET25* mRNA accumulated in cells grown in the presence of 0.1 mM AdoMet (25). The results reported here are in accord with these previous findings, as we have strong evidence that *O*-acetylhomoserine sulfhydrylase synthesis is regulated not only by modulation of mRNA synthesis but also by a superimposed regulation probably targeted at the 5′ region of the *MET25* mRNA. Under conditions in which this regulation is acting (0.1 mM AdoMet), the untranslated mRNA could accumulate in the cell (25) and be readily translated when repression is relieved.

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