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The expression of the *MET25* gene of *Saccharomyces cerevisiae* is regulated transcriptionally

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Summary. The *MET25* gene of *Saccharomyces cerevisiae* was cloned by functional complementation after transformation of a yeast *met25* mutant. Subcloning of the DNA fragment bearing *MET25* located the gene on a 2.3 kb region. The gene was formally identified by integration at the chromosomal *MET25* locus.

The cloned *MET25* gene was used as a probe to measure the *MET25* messenger RNA in a wild-type strain grown under conditions which promoted or failed to promote repression of *MET25* expression. It was found that, under repression conditions, *MET25* messenger RNA was reduced tenfold when compared with non-repression conditions. This suggests that the expression of *MET25* is regulated transcriptionally. The direction of transcription, the size of the transcript and the position of the transcribed part of the gene were determined. Deletion mapping of the regulatory region was carried out. Deleted plasmids were introduced back into yeast cells and tested for their ability to complement *met25* mutations and to promote regulation of expression of the *MET25* gene by exogenous methionine. By this method the regulatory region was found to be confined to a 130 bp region.

Introduction

In *Saccharomyces cerevisiae*, we have studied the regulation of methionine biosynthesis extensively (Cherest et al. 1973a, b; Antoniewski and de Robichon-Szulmajster 1973). Our data show that methionine biosynthesis is regulated only by repression of enzyme synthesis and that at least five enzymes are subject to the same regulatory system. As the genes encoding these enzymes are unlinked we decided to study at the molecular level the regulatory system acting on the expression of these unlinked genes. We have already shown that the expression of the *MET3* gene encoding ATP sulphurylase is regulated transcriptionally (Cherest et al. 1985). Here we report the cloning and the study of the expression of the *MET25* gene which encodes O-acetyl homoserine sulphydrylase (OAH sulphydrylase). It must be noted here that mutants lacking OAH sulphydrylase activity have been called *met8* mutants (Mortimer and Hawthorne 1966; Cherest et al. 1969). It was shown later that different *met8* mutants complement each other, and so the gene encoding OAH sulphydrylase was renamed *MET25* (Masselot and de Robichon-Szulmajster 1975).

In bacteria, cysteine is synthesized by sulphydrylation of serine and is then used as a precursor of methionine synthesis via the formation of cystathionine and its cleavage into homocysteine (Kaplan and Flavin 1966; Kredich and Tomkins 1966) which is then methylated to give methionine. On the basis of the Met^- phenotype of *met25* mutants, Cherest et al. (1969) had concluded that, in *S. cerevisiae*, homocysteine results directly from sulphydrylation of O-acetyl-homoserine. However, in view of the identical growth of *met25* mutants on cysteine- or homocysteine-supplemented media, it was assumed that cysteine can be used, in some conditions, as a precursor for homocysteine formation (Masselot and de Robichon-Szulmajster 1975). It was shown later that OAH sulphydrylase also catalyze the sulphydrylation of O-acetyl-serine (OAS) into cysteine (Yamagata et al. 1974; Yamagata 1976). This finding reopened the question about the de novo synthesis of homocysteine by *S. cerevisiae* because in *met25* mutants the loss of the two enzymatic activities results in the interruption of both pathways, i.e. formation of homocysteine from cysteine via cystathionine or direct synthesis by sulphydrylation of O-acetyl-homoserine. The isolation of mutants growing only on homocysteine or only on cysteine could solve this problem. A recent report (Ono et al. 1984) presents some evidences that both pathways could be operational in *S. cerevisiae* as they are in *Saccharomycopsis lipolitica* (Morzycka and Paszewski 1979) and in *Aspergillus nidulans* (Paszewski and Grabski 1975). This agrees completely with the growth pattern and the enzymatic defects exhibited by *met25* mutants.

It has been reported by Yamagata et al. (1975) that strains 13 and 17 are devoid of OAH-OAS sulphydrylase activity. These strains (Na13 and Na17) have been classified as *met17* mutants by Masselot and de Robichon-Szulmajster (1975) who have shown moreover that *met17* mutants can grow on media supplemented with any of the following metabolites: sulphite, sulphide, homocysteine, cysteine or methionine. This growth pattern is not compatible with a non-functional OAH-OAS sulphydrylase. Later, we showed that, in vitro, *met17* mutants have no 3′ phosphoadenosine 5′ phosphosulphate (PAPS) reductase activity and, as found by Yamagata et al. (1975), a very low OAH-OAS sulphydrylase activity. Nevertheless, multiple enzy-

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Abbreviations: kl, Kilobase; bp, base pair

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matic defects in monogenic *Met*$^-$ mutants seem to be the rule (Masselot and Surdin-Kerjan 1977). We thus think that *MET25* is the structural gene of OAH-OAS sulphydrylase because *met25* mutants exhibit a growth pattern compatible with this defect and no OAH-OAS sulphydrylase activity in vitro. On the contrary, because *met17* mutants are able to grow on sulphide they must synthesize enough OAH-OAS sulphydrylase in vivo for methionine and cysteine synthesis. Therefore we think that, for example, the *MET17* gene product could interfere in some way with OAH-OAS sulphydrylase and PAPS reductase activities.

However, the key role of the *MET25* gene is assessed by the fact that its expression is co-regulated by exogenous methionine with that of other *MET* genes so we decided to clone *MET25* in order to continue our study of the regulation of these genes.

Materials and methods

Yeast and bacterial strains. Strains used in this work and their sources are listed in Table 1.

Media. For *Escherichia coli* the medium used was LB medium (Miller 1972). When necessary, ampicillin (Ap; 40 μg/ml) and/or tetracycline (Tc; 5 μg/ml) were added.

For yeast, minimal medium was prepared with Difco Yeast Nitrogen base without amino acids (0.7%) supplemented with 2% glucose (YNB medium). When necessary, uracil (10 μg/ml), L-histidine (200 μg/ml) and DL-leucine (50 μg/ml) were added; DL-methionine was added at 0.2 mM for auxotrophic strains and at 2 mM to promote repression of enzyme synthesis. Before transformation, yeast cells were grown on complete YPGA medium (yeast extract 0.5%, peptone 0.5%, glucose 3% and adenine 20 μg/ml).

Yeast cell free extracts and enzyme assay. Yeast cell free extracts were prepared as described by Cherest et al. (1973a). O-acetyl homoserine (OAH) sulphydrylase (O-acetyl homoserine sulphydrylase, EC 4.2.99.8) was assayed as described by Wiebers and Garner (1967) and the homocysteine formed in the reaction was estimated by the method of Kredich and Tomkins (1966). O-acetyl serine (OAS) sulphydrylase was assayed as described by Yamagata et al. (1974) and the cysteine formed in the reaction was also measured by the method of Kredich and Tomkins (1966). Protein concentration was measured by the biuret method (Gornall et al. 1949) with bovine serum albumine as reference.

Permeabilized cells. Cells were permeabilized as described by Magee and de Robichon-Szulmajster (1968). The dry weight per ml was related to the number of cells in the same volume.

Plasmids. The plasmids used were pBR322 (Bolivar et al. 1977) and the shuttle plasmid pFL1 (Chevallier et al. 1980). The yeast genomic library used was constructed as described by Loison et al. (1981) except that yeast DNA fragments were generated by partial digestion with *Sau*3A.

DNA preparations. The preparation of plasmid DNA from *E. coli* cultures was performed as described by Clewell and Helinski (1972). A second caesium chloride-ethidium bromide equilibrium centrifugation was performed on plasmid preparations. For rapid analysis, the alkaline extraction procedure described by Birnboim and Doly (1979) was used. Plasmid transfer from yeast to *E. coli* was performed as described by Crabeel et al. (1981) although the yeast cells were grown overnight in 10 ml of liquid selective minimal medium.

Table 1. Bacterial and yeast strains

Organism	Strain	Genotyp	Source
Escherichia coli	C600	*thr, met, recBC,* r^-m^-	A. Campbell
Saccharomyces cerevisiae	FL100 CC365-7A	a *ura3-251, ura3-373, his3, leu2, met25*	F. Lacroute H. Cherest

Transformation. Transformation of *S. cerevisiae* was as described by Hinnen et al. (1978). The spheroplasts were prepared from early logarithmic phase cells (1 to 3×10^6 cells/ml) with zymolyase 60,000 (5 μg/ml). Transformation of *E. coli* was as described by Cohen et al. (1972).

Restriction, ligation and agarose gel electrophoresis. Restriction and ligation were performed as indicated by the suppliers of restriction endonucleases and of T4 DNA ligase.

Electrophoresis of DNA fragments was carried out routinely in 0.8% agarose gels in 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA (TEB buffer) using a horizontal slab gel apparatus. Fragments of bacteriophage ϕ80 DNA generated by *Bam*HI, *Alu*I-generated fragments of plasmid pBR322 and *Hin*dIII-generated fragments of bacteriophage λ DNA were used as molecular weight standards.

Preparation of yeast RNA. Yeast RNA was prepared and the enriched poly(A)$^+$ RNA was isolated as described by Cherest et al. (1985).

Measurement of the rate of transcription. The rate of transcription in vivo was measured as described by Cherest et al. (1985). The probe was the *Eco*RI-*Hin*dIII fragment of plasmid pM25-7 (see Fig. 2) purified from low-melting-point agarose gels (Wieslander 1979).

Northern blots and S1 mapping. Northern blots and S1 mapping were performed as in Cherest et al. (1985).

Deletions in the 5′ region of the MET25 gene. Plasmid pM25-7 (Fig. 2) was restricted by *Bam*HI. It was then digested by the exonuclease Bal31 for different times (empirically determined), treated by the Klenow fragment of *E. coli* DNA polymerase I and recircularized by T4 DNA ligase in the presence of a phosphorylated *Bam* linker, as described by Maniatis et al. (1982). The *Bam*HI-*Sal*I fragments of the deleted plasmids were then reinserted in place of the *Bam*HI-*Sal*I fragment of plasmid pFL1 (Fig. 1). The resulting plasmids were used to transform *E. coli* to Ap resistance and purified from transformed *E. coli* strains for further studies.

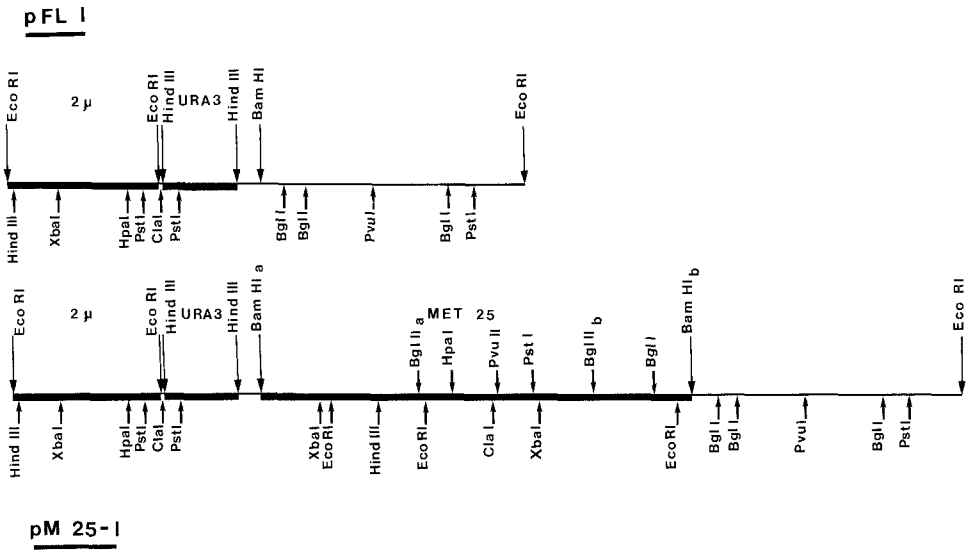

Fig. 1. Restriction maps of plasmids pFL1 and pM25-1. *Thin lines* represent plasmid pBR322 DNA and *thick lines* represent yeast DNA

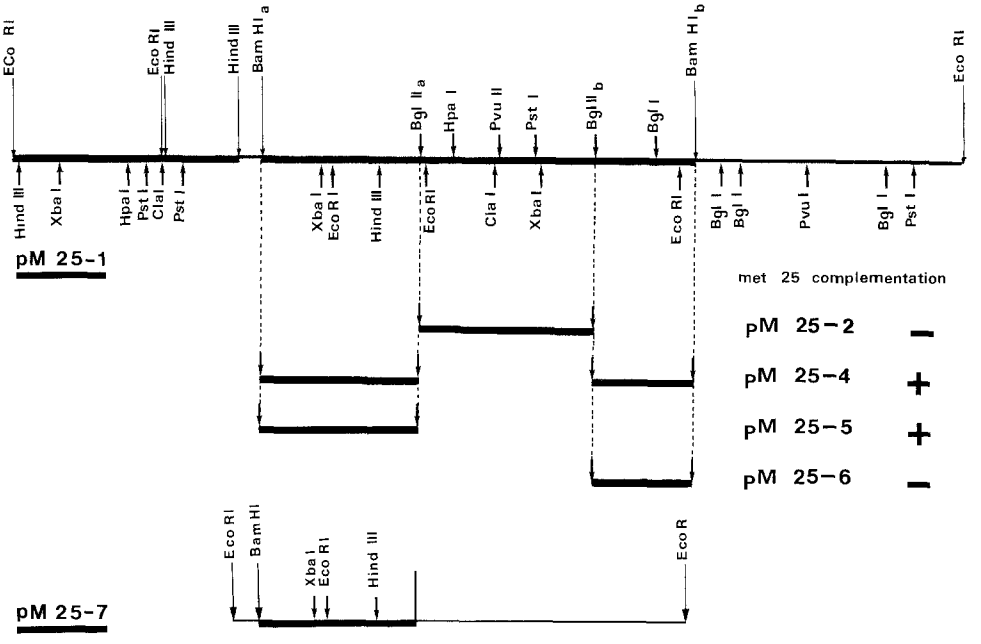

Fig. 2. Localization of the *MET25* gene. Fragments of plasmid pM25-1 were recloned in the *Bam*HI site of plasmid pFL1, generating the plasmids shown under pM25-1. *Gaps* represent deleted yeast DNA. The structure of the different plasmids was verified by restriction endonuclease digestions and each plasmid was tested for its ability to transform strain CC365-7A to Met^+ and Ura^+. +, capacity to transform CC365-7A to Met^+ and Ura^+; −, lack of this capacity

Results

Isolation of a plasmid able to complement a mutation in the MET25 gene

We isolated a chimaeric plasmid bearing the *MET25* gene by functional complementation of a *met25* mutation in *S. cerevisiae*. Plasmid DNA (45 μg), from a yeast genomic library constructed as described in Materials and methods, was used to transform strain CC365-7A to the Ura^+, Met^+ phenotype (the *ura3* mutations of strain CC365-7A are complemented by the *URA3* gene carried by plasmid pLF1). Eight independent Ura^+ Met^+ transformants were obtained. The nucleic acid from these eight transformants was extracted and used to transform the bacterial strain C600 to Ap resistance. The nucleic acid extracts of only two yeast transformants gave rise to Ap-resistant *E. coli* transformants and the plasmids the latter harboured were purified. These two plasmids had different restriction endonuclease sites and were used to transform the yeast strain CC365-7A to Met^+ or to Ura^+ in two different selection experiments. Only one plasmid gave the same number of transformants in the two selections; moreover the transformants selected as Met^+ were simultaneously Ura^+ and vice/versa. This plasmid, named plasmid pM25-1 was further studied.

Structure of plasmid pM25-1

Fragments of plasmid pM25-1 were generated by single or double digestion by restriction endonucleases. The size of the fragments was analysed by electrophoresis on 0.8% agarose. Results are shown in Fig. 1 with plasmid pFL1 for comparison. Plasmid pM25-1 carries a yeast DNA fragment (6.35 kb) complementing the *met25* mutation of strain CC365-7A.

Table 2. Genetic analysis of the integration of the *MET25* and *URA3* genes

Cross	Number of asci analysed	Type of asci for					
		Methionine			Uracil		
		$4^+/0^-$	$3^+/1^-$	$2^+/2^-$	$4^+/0^-$	$3^+/1^-$	$2^+/2^-$
CC400[a]	14	14			4	6	4
CC401[b]	13	13			2	10	1

[a] CC400, strain FL100 × strain 2 (α, *his3*, *leu2* bearing the integrated plasmid pM25-8)
[b] CC401, strain FL100 × strain 6 (α, *his3*, *leu2*, bearing the integrated plasmid pM25-8)

Table 3. Expression of the cloned *MET25* gene in a yeast strain

Strain	Supplements to minimal medium[a]	Specific activity[b]	
		OAH sulphhydrylase	OAS sulphhydrylase
FL100	–	120	10
	Met	30	3.5
CC365-7A	–	10	1.7
	Met	10	1.0
CC365-7A (pM25-1)	–	684	40
	Met	74	5.8
CC365-7A (pM25-5)	–	660	72
	Met	100	10

[a] For strain FL100 minimal medium was YNB medium described in Materials and methods. For strain CC365-7A minimal medium was YNB medium supplemented with histidine and leucine when a plasmid was present and histidine, leucine, uracil and methionine when it bore no plasmid. Concentrations are given in Materials and methods. Met, DL-methionine (2 mM) was added
[b] Expressed in nmoles × min^{-1} × mg $protein^{-1}$

Sub-cloning of plasmid pM25-1

Sub-cloning of plasmid pM25-1 was carried out as drawn schematically in Fig. 2 and the different plasmids obtained were tested for their ability to transform strain CC365-7A to Met^+ and Ura^+.

We obtained plasmid pM25-5, able to transform strain CC365-7A and carrying the 2.3-kb *Bam*HI_a – *Bgl*II_a fragment of plasmid pM25-1. This yeast DNA fragment was inserted in plasmid pBR322; the resulting plasmid, pM25-7 was used as a probe in a number of experiments (Fig. 2).

Integration of the cloned putative MET25 gene into chromosomal DNA

In order to identify the plasmid-borne *MET25* gene, it was necessary to integrate it into chromosomal DNA and to show that this had occurred at the *MET25* locus. For this purpose an integrative plasmid was constructed by eliminating the 2 μ fragment from plasmid pM25-5 to give pM25-8. This plasmid was cut with *Xba*I to enhance the frequency of integration at the *MET25* locus and used to transform strain CC365-7A to Ura^+, Met^+. Two of the transformants (strains 2 and 6) were crossed to the wild-type strain FL100 after their phenotype was shown to be stable. The two diploid strains (CC400 and CC401) were sporulated and the segregation of the different characters was studied. The results (Table 2) showed that all ascospores were Met^+ while some Ura^- spores were recovered and therefore, that the genes carried by plasmid pM25-8 were integrated not at the *URA3* locus but at the *MET25* locus, since otherwise Met^- spores would have been recovered. The His^- and Leu^- phenotypes segregated as expected (results not shown).

Expression of the cloned MET25 gene in yeast

We have measured the specific activity of OAH sulphydrylase in a wild-type strain and in strain CC365-7A transformed by plasmid pM25-1 or pM25-5. The results (Table 3) showed that the specific acitivity of OAH sulphydrylase was increased five- to sixfold in the transformed strains as compared with the wild-type strain. It had been shown by Yamagata et al. (1974) that the OAH and OAS sulphydrylase activities are borne by the same protein, so, we also measured OAS activity and found that it increased four- to sevenfold in transformed strains as compared with the wild-type strain. We did not measure the number of plasmids per cell in this experiment. Nevertheless, Cherest et al. (1985) have shown that the same plasmid vector bearing the *MET3* gene is present in a yeast strain at low copy number (five to eight per cell). We therefore think that the specific activity increase reported here reflects the number of plasmids present.

Moreover, growth in the presence of 2 mM DL-methionine, which promotes repression of OAS-OAH sulphydrylase synthesis in the wild-type strain, also causes repression of the synthesis of these enzymes in the transformed strains, showing that the *MET25* gene has been cloned with all the sequences necessary for regulation to take place (Table 3).

Transcription of the MET25 gene during growth under repressing or non-repressing conditions

In order to determine at which level the repression of synthesis of OAH-OAS sulphydrylase takes place, we measured the level of *MET25* mRNA in different strains after growth in minimal medium or in minimal medium containing DL-methionine at a concentration which promotes repression. The mRNAs were labelled with ^3H-adenine and hybridized to a *MET25* probe loaded on nitrocellulose circles. The results are shown in Table 4. After growth in the presence of methionine the level of *MET25* mRNA is about 10% of that found in cells grown in the absence of methionine. This decrease in the level of *MET25* mRNA observed after growth in the presence of methionine could be due either to a decrease in the *MET25* mRNA rate of synthesis or to a decrease in *MET25* mRNA stability. Thus, we measured the stability of *MET25* mRNA when the cells were incubated with or without methionine. The results (Fig. 3) show that the decay rate of *MET25* mRNA was the same under the two conditions and a half life of 10 ± 1 min was determined. These results show unambiguously that the regulation of expression of the *MET25* gene takes place at transcription as growth under repressing conditions leads to a decrease in the rate of synthesis of *MET25* mRNA.

Table 4. Transcription in vivo of the *MET25* gene

Strain[a]	Supplements to minimal medium[b]	RNA added (μg)	RNA specific activity[c]	Hybridization			OAH sulphydrylase activity[f]
				cpm hybridized[d]		Specific hybridization[e]	
				Total	per μg of RNA added		
FL100	–	6.7	4.6×10^4	174	26	5.6×10^{-4}	123
	Met	5.7	2.9×10^4	6	1	3.6×10^{-5}	42
CC365-7A	–	2.4	3.1×10^4	531	221	7.0×10^{-3}	828
(pM25-5)	Met	3.2	3.5×10^4	170	53	1.5×10^{-3}	117

[a] The plasmid borne by strain CC365-7A is indicated in parentheses
[b] Minimal medium, see legend of Table 3; Met, DL-methionine (2 mM) was added
[c] RNA specific activity is cpm/μg measured as TCA-precipitable material
[d] Labelled RNA from either strain was hybridized to plasmid pM25-16 on nitrocellulose discs. The results given as the cpm hybridized to plasmid pM25-16 minus the cpm hybridized to plasmid pBR322
[e] Ratio of the cpm specifically bound to *MET25* per μg of added RNA to the RNA specific activity
[f] In nmoles $\times$ min^{-1} $\times$ mg protein^{-1}

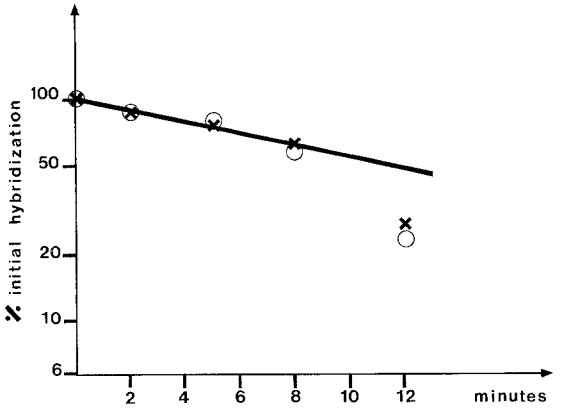

Fig. 3. Decay of *MET25*-specific mRNA. RNA was pulse labelled as indicated in Materials and methods for the measurement of the rate of transcription. Labelled cells were separated from the medium, washed and suspended in YNB medium and in YNB medium containing 2 mM DL-methionine. RNA was extracted from samples taken at intervals and hybridized to plasmid pM25-16 (see Fig. 8) as indicated in Materials and methods. Specific hybridization is calculated as in Table 4 and is expressed as percent of initial hybridization. o, RNA extracted from cells incubated in YNB medium; ×, RNA extracted from cells incubated in YNB-methionine medium

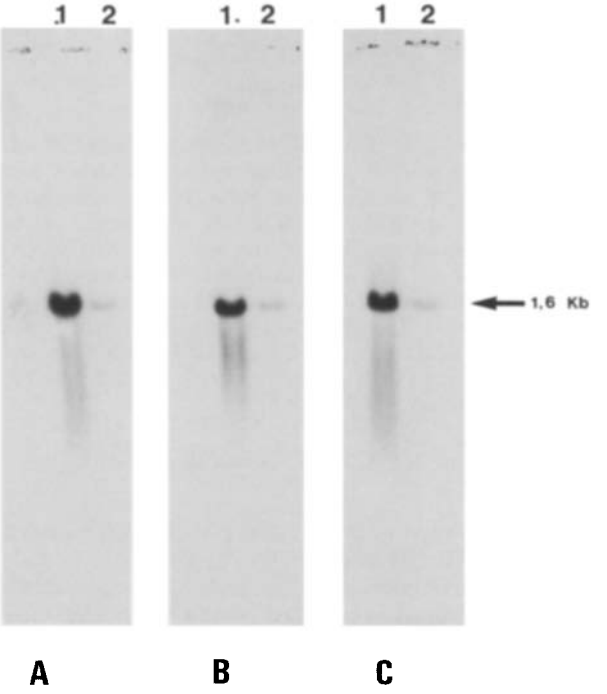

Fig. 4A–C. Northern blot of enriched poly(A)$^+$RNA extracted from the transformed strain CC365 grown in the presence or absence of methionine. Poly(A)$^+$RNA (30 μg) extracted from CC365-7A bearing plasmid pM25-5 was submitted to electrophoresis. The probes used in hybridization were: **A** *Bam*HI-*Eco*RI fragment of pM25-7 (Fig. 2); **B** *Eco*RI-*Hin*dIII fragment of pM25-7; **C** *Hin*dIII-*Hpa*I fragment of pM25-7. Lane 1, poly(A)$^+$RNA was extracted from CC365-7A bearing plasmid pM25-5 and grown in minimal medium (see legend of Table 3). Lane 2, poly(A)$^+$RNA was extracted from CC365-7A bearing plasmid pM25-5 and grown in minimal medium containing 2 mM DL-methionine

In addition, the results in Table 4 show that, in the transformed strain CC365-7A bearing plasmid pM25-5 grown in minimal medium, the level of *MET25* mRNA is tenfold higher than in the wild-type strain grown under the same conditions. This is compatible with the sixfold increase in the OAH sulphydrylase activity observed in this experiment. The decrease in the level of *MET25* mRNA after growth under repressing conditions can also be seen in transformed cells.

Northern blot analysis

Enriched poly(A)$^+$ RNA extracted from strain CC365-7A bearing plasmid pM25-5 was treated and submitted to electrophoresis as described in Materials and methods. Plasmid pM3-1 (Cherest et al. 1985) digested by *Hin*dIII and denatured under the same conditions as the RNA samples was used as a molecular weight standard. The part of the gel bearing the standard was stained with ethidium bromide and the poly(A)$^+$ RNA was hybridized to three different probes. In each case only one *MET25* mRNA, of $1{,}600 \pm 50$ nucleotides, was detected (Fig. 4). After growth in the presence of 2 mM methionine hardly any mRNA hybridized to the probes, showing once more that the regulation of *MET25* expression is transcriptional.

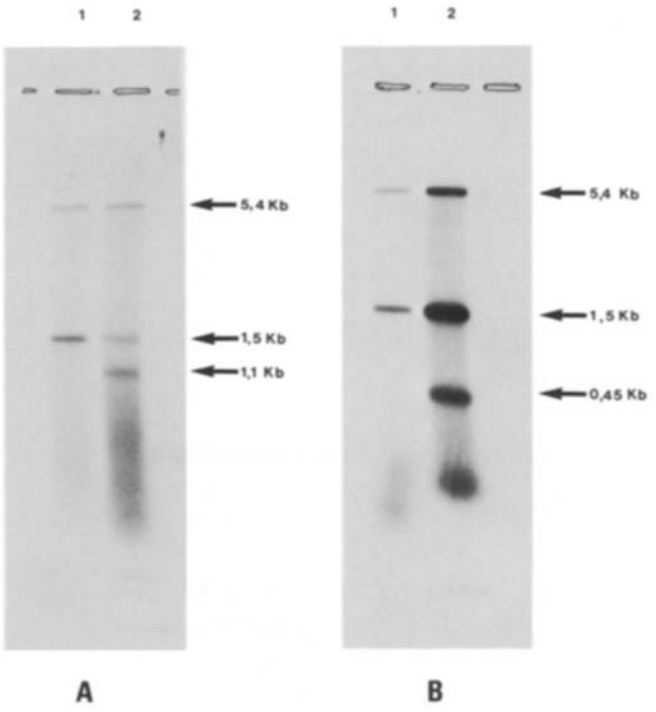

Fig. 5A, B. S1 mapping of the *MET25* gene. Plasmid pM25-7 was cut by *Eco*RI and labelled, hydridized to poly(A)$^+$RNA and treated by nuclease S1 as indicated in Materials and methods. **A** *Eco*RI site labelled at the 3′ end; **B** *Eco*RI site labelled at the 5′ end. Lane 1, control sample (with no added poly(A)$^+$RNA) treated by nuclease S1. The two bands are the 5.4 and 1.5 kb DNA fragments obtained by digestion of pM25-7 by *Eco*RI (see Fig. 2). Lane 2, sample hybridized to poly(A)$^+$RNA and treated by nuclease S1. In addition to the 5.4 and 1.5 kb bands one can see the DNA fragment protected from nuclease S1 action by poly(A)$^+$RNA: 1.1 kb in **A** and 0.45 kb in **B**

Mapping position of MET25 mRNA and direction of transcription

For S1 mapping experiments, plasmid pM25-7 was digested with *Eco*RI or *Hin*dIII and labelled at the 3′ or 5′ ends as indicated in Materials and methods. The radioactive DNAs were hybridized to enriched poly(A)$^+$RNA extracted from the wild-type strain FL100 grown in minimal medium. After hybridization the samples were treated with nuclease S1 and the S1-resistant RNA-DNA hybrid was analysed by 1% alkaline agarose gel electrophoresis.

When the *Eco*RI site was labelled at the 3′ end we found an S1-resistant 1,100 ± 50 bp RNA-DNA hybrid but when it was labelled at the 5′ end, we found an S1-resistant 450 ± 50 bp RNA-DNA hybrid (Fig. 5). With the *Hin*dIII site labelled at the 5′ end an S1-resistant 1,100 ± 50 bp RNA-DNA hybrid was found (Fig. 6). Comparing these results with the restriction map of plasmid pM25-7 we concluded that the transcribed part of the gene and the direction of transcription are as shown in Fig. 7.

Thus, in these S1 mapping experiments we have found a transcribed region of 1,550 ± 100 bases which is very close to the length of the *MET25* mRNA found in Northern blot experiments (1,600 ± 50 nucleotides).

Deletion mapping of a regulatory region

We have shown that the modulation of *MET25* gene expression is transcriptional. We thought we could thus identify

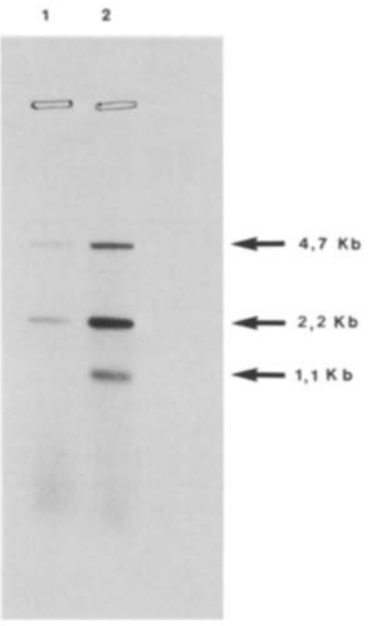

Fig. 6. S1 mapping of the *MET25* gene. Plasmid pM25-7 was cut by *Hin*dIII and labelled at the 5′ end. Lane 1, control sample (see Fig. 5 legend). The two bands are the 4.7 and 2.2 kb fragments obtained by digestion of pM25-7 by *Hin*dIII (Fig. 2). Lane 2, sample hybridized with poly(A)$^+$RNA and treated with nuclease S1. One can see the additional 1.1 kb fragment protected from nuclease S1 action by poly(A)$^+$RNA

Table 5. Specific activities of OAH and OAS sulphydrylase in strains bearing *MET25* plasmids

Strain[a]	Supplements to minimal medium[b]	Specific activities[c]	
		OAH sulphydrylase	OAS sulphydrylase
FL100	–	120	20
	Met	30	10
CC365-7A	–	10	3
	Met	10	3
CC365-7A (pM25-5)	–	660	60
	Met	100	16
CC365-7A (pM25-9)	–	700	54
	Met	100	15
CC365-7A (pM25-10)	–	680	56
	Met	100	13
CC365-7A (pM25-12)	–	700	52
	Met	100	16
CC365-7A (pM25-14)	–	720	54
	Met	100	12
CC365-7A (pM25-15)	–	650	48
	Met	620	46

[a] The plasmids borne by strain CC365-7A are indicated in parentheses

[b] Minimal medium, see legend of Table 3; Met, DL-methionine (2 mM) was added

[c] Expressed in nmoles × min^{-1} × mg protein^{-1}

the *cis*-acting element necessary for this regulation. As the direction of transcription is from *Bam*HI towards *Sal*I, plasmid pM25-7 was cut at the *Bam*HI site, digested by the exonuclease Bal31, and treated as described in Materials and methods. We obtained several plasmids which trans-

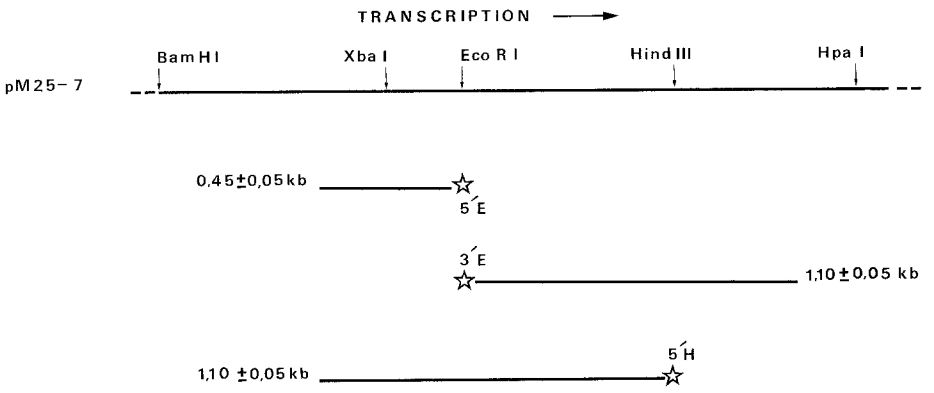

Fig. 7. Direction of transcription of the *MET25* gene. The results of S1 mapping experiments are shown schematically. The direction of transcription is shown by the *arrow*

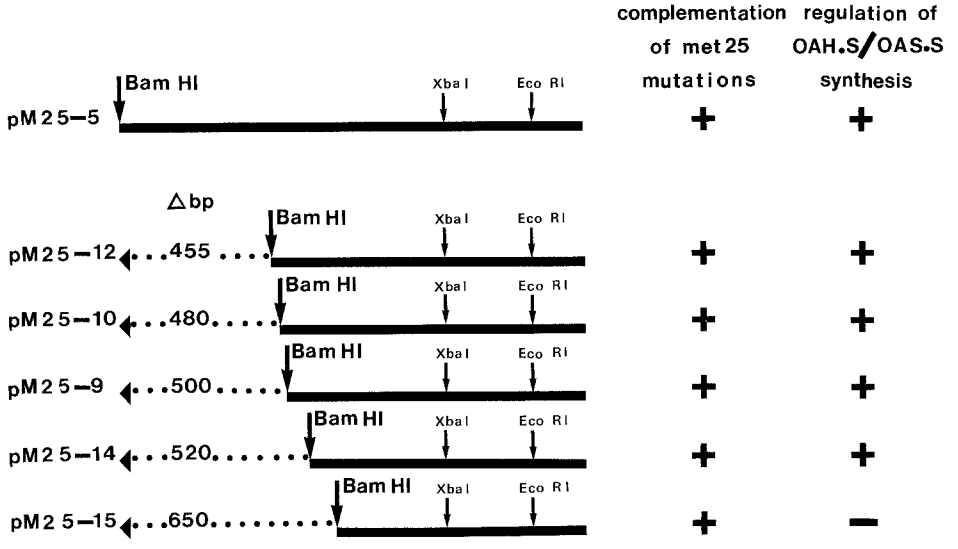

Fig. 8. Deletion mapping of a regulatory region in the *MET25* gene. Deletions were generated from the *Bam*HI site of pM25-5 by digestion with Bal31 exonuclease as described in Materials and methods. The *thick line* represents the *MET25* region DNA. The *dotted lines* represent the deletions. The ability of the different deleted plasmids to transform CC365-7A to Met^+ and Ura^+ is indicated by +. The regulation of the expression of the OAH-OAS sulphydrylase (OAH/OAS-S) encoded by the plasmid is indicated by + when it is repressed by growth in the presence of 1 mM methionine and by − when this repression is absent

formed CC365-7A to Met^+, Ura^+ and seven transformants were tested for their OAH-OAS sulphydrylase activity. One transformant was found to have retained enzymatic activity (Table 5) but showed no repression of OAH-OAS sulphydrylase synthesis after growth in the presence of methionine. The restriction maps of the plasmids present in these transformants were established and are shown in Fig. 8. It can be seen that pM25-15 which bears a non-regulated *MET25* gene shows a 130-bp deletion when compared with the shortest plasmid which bears a regulated *MET25* gene (pM25-14).

Discussion

We have cloned the *MET25* gene of *S. cerevisiae* and identified this gene by integration at the *MET25* chromosomal locus. The *MET25* gene is transcribed into a polyadenylated mRNA 1,600 nucleotides long. Yamagata (1976) has shown that the molecular weight of the subunit of the OAH-OAS sulphydrylase is 50,000 daltons and that the number of aminoacid residues is 468. A messenger RNA 1,600 nucleotides long is in good agreement with these results, which is further evidence in favour of *MET25* being the structural gene for OAH-OAS sulphydrylase. S1 mapping experiments show that the transcribed part of the gene is entirely located within the cloned fragment. The good agreement between the results of S1 mapping and of Northern blot hybridization show that no intervening sequences are present in the *MET25* gene although we cannot exclude short untranscribed regions near the end of the gene.

Growth in the presence of methionine, which leads to 80% repression of OAH-OAS sulphydrylase synthesis, also results in a 90% decrease in the level of *MET25* mRNA. As this is not due to a decrease in *MET25* mRNA stability we conclude that the regulatory system controlling *MET25* expression acts on transcription. This result corroborates the results obtained on the expression of *MET3*, a gene which is co-regulated with *MET25*.

The deleted plasmids described here are of particular interest as the phenotype they confer has been studied in vivo in growth conditions which do or do not lead to repression of *MET25* expression. By this method we have determined a 130-bp region preceding the transcribed part of the gene which is necessary for regulation to take place. We are now trying to identify in this region the *cis*-acting regulatory sequence. This is also being done with the *MET3* gene in order to compare these sequences in the two co-regulated genes.

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