Transcriptional regulation of methionine synthase by homocysteine and choline in Aspergillus nidulans

Magdalena M. KACPRZAK*1, Irmina LEWANDOWSKA*1, Rowena G. MATTHEWS† and Andrzej PASZEWSKI*2

*Department of Genetics, Institute of Biochemistry and Biophysics, PAS, Pawinskiego 5a, 02-106 Warsaw, Poland, and Biophysics Research Division and Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109, U.S.A.

Roles played by homocysteine and choline in the regulation of MS (methionine synthase) have been examined in fungi. The Aspergillus nidulans metH gene encoding MS was cloned and characterized. Its transcription was not regulated by methionine, but was enhanced by homocysteine and repressed by choline and betaine. MS activity levels were regulated in a similar way. The repression by betaine was due to its metabolic conversion to choline, which was found to be very efficient in A. nidulans. Betaine and choline supplementation stimulated growth of leaky metH mutants apparently by decreasing the demand for methyl groups and thus saving methionine and S-adenosylmethionine. We have also found that homocysteine stimulates transcription of MS-encoding genes in Saccharomyces cerevisiae and Schizosaccharomyces pombe.

Key words: betaine, choline, homocysteine, methionine synthase gene, regulation.

INTRODUCTION

MS (methionine synthase) catalyses the formation of methionine by the transfer of a methyl group from methyltetrahydrofolate to homocysteine. The homocysteine methylation reaction is the point of convergence of two metabolic domains, i.e. sulphur metabolism and one-carbon unit metabolism. This reaction is the last step in methionine biosynthesis and, indirectly, also serves, among others, to generate the methyl group of SAM (S-adenosylmethionine), a substrate required for more than a hundred biological methylation reactions.

Two types of this enzyme are found: cobalamin-dependent MS (EC 2.1.1.13) and cobalamin-independent MS (EC 2.1.1.14), which share no similarity and have different evolutionary origins [1]. The former type is found in bacteria, protozoa and metazoa. Bacteria and protozoa may also possess cobalamin-independent MS, which is the only enzyme found in fungi and plants.

Cobalamin-independent MS uses mainly a triglutamate derivative of methyltetrahydrofolate as a methyl group donor. The GenBank® database contains more than 40 sequence codes for these enzymes. However, the regulation of their synthesis has been studied in relatively few organisms.

The Escherichia coli MS encoded by the metE gene is regulated by the MetR transcription factor, which is activated by homocysteine or homocysteine thiolactone [2,3]. It is repressed by the metF gene product with SAM acting as a co-repressor [4]. In plants, the MS level varies in different organs and changes at day/night periods [5,6]. An increase in the MS transcript, but not protein level, was observed in Catharanthus roseus and Solanum tuberosum under stress induced by sucrose [6,7]. Interestingly, in Chlamydomonas reinhardtii, cobalamin-independent MS was induced severalfold during adhesion-induced activation of gametes, which involves a cAMP-dependent cascade of signal transduction [8]. This may result from an increased demand for SAM (and also for methionine) for carboxy methylation of signalling molecules and for the synthesis of polyamines involved in chromatin restructuring.

Among fungi, methionine synthesis has been most extensively studied in Saccharomyces cerevisiae. The MS-encoding gene MET6 [9] is repressed in the presence of methionine in the growth medium, but apparently by a different mechanism when compared with that regulating other MET genes [10,11]. Moreover, the enzyme level is significantly decreased after osmotic [12] and oxidative [13] shocks. In contrast with S. cerevisiae, MS is up-regulated under heat conditions and during the yeast-to-hyphal transition in the human pathogen Candida albicans [14]. Burton and Metzenberg [15] observed slightly increased levels of MS in Neurospora crassa mutants defective in SAM or polyglutamate synthesis. The enzyme level was decreased when growth medium was supplemented with choline.

In Aspergillus nidulans, despite extensive studies of sulphur amino acid metabolism, no investigation of an MS gene and regulation of its expression have been performed so far. In this fungus, biosynthesis and metabolism of sulphur amino acids comprises a rich combination of metabolic options (see Figure 1, reviewed in [16]). Several sulphur metabolic genes, particularly those involved in sulphate assimilation, are regulated by a cascade of negatively acting scon (sulphur controller) genes [17] and the positively acting metR gene, encoding a sulphur-specific transcription factor [18] forming the cysteine-mediated SMR (sulphur metabolite repression) system. The scon mutations render sulphur metabolism insensitive to SMR, whereas metR mutants are methionine-requiring auxotrophs.

Two mutants, metH2 and metH10 (formerly metD10), growing on methionine but not on homocysteine, apparently blocked in the homocysteine methylation step, have been known for many years [19]. The mutants contain twice as much cystathionine as the wild-type strain and accumulate some homolanthionine, a higher homologue of cystathionine [20], which results from an increased homocysteine pool. Genetic tests pointed to a very close
The conditional metH gene was localized on chromosome III [28], the metH2, argB2, nicA2, biaI, ya2 strain was co-transformed with DNA isolated from a fraction of the A. nidulans cosm id chromosome-specific library [29] covering this chromosome (divided into subpools) and the Arp1 helper plasmid, which increases transformation efficiency as much as 200-fold. The subpool that gave met+ transformants was further subdivided and one cosm id (W25A01) restoring methionine prototrophy was found. It was digested with several restriction endonucleases and used for subsequent transformation. The cosm id digested with XbaI gave the highest number of transformants. Individual DNA fragments were isolated from agarose gel and used for transformation. Finally, a 5.1 kb XbaI–XbaI-transforming fragment was cloned into the pUC19 vector giving pLX2. This fragment was sequenced on both strands using several subclones and standard primers for pUC19 and pBluescriptIIKS+ vectors.

Transformation of A. nidulans

Transformation of A. nidulans was performed as described by Sienko et al. [27].

Cloning and sequencing of metH gene

Since the metH gene was isolated from chromosome III [28], the metH2, argB2, nicA2, biaI, ya2 strain was co-transformed with DNA isolated from a fraction of the A. nidulans cosm id chromosome-specific library [29] covering this chromosome (divided into subpools) and the Arp1 helper plasmid, which increases transformation efficiency as much as 200-fold. The subpool that gave met+ transformants was further subdivided and one cosm id (W25A01) restoring methionine prototrophy was found. It was digested with several restriction endonucleases and used for subsequent transformation. The cosm id digested with XbaI gave the highest number of transformants. Individual DNA fragments were isolated from agarose gel and used for transformation. Finally, a 5.1 kb XbaI–XbaI-transforming fragment was cloned into the pUC19 vector giving pLX2. This fragment was sequenced on both strands using several subclones and standard primers for pUC19 and pBluescriptIIKS+ vectors.

cDNA isolation and splicing analysis

A cDNA copy of the metH gene was isolated from the A. nidulans λ Zap cDNA library (constructed by R. Aramayo, Fungal Genetics Stock Center, Kansas City, KS, U.S.A.) using UNI-ZAP!!!XR Cloning kit (Stratagene). Plaque hybridization with DIG-labeled probe was performed using Dig High Prime DNA-labeling kit (Roche, Rotkreuz, Switzerland) according to the manufacturer’s instructions. A PCR fragment, obtained with the following primers [25], was used as a probe. DNA inserts in pBluescriptIIKS were excised from the λ Zap plasmid containing the AlcA ethanol inducible promoter [25] and the pyr4 marker which complements A. nidulans pyrG89 mutation. The resulting pAH41 plasmid was transformed into pyrG89, pabaA1 and integrated into the metH locus giving a conditional, ethanol-dependent metH+ mutant.

A. nidulans strains were grown on MM (minimal medium) or complete medium as described by Cove [26] for 16–18 h at 37 °C with shaking. Solid media contained 1.5% (w/v) agar. Supplements added to the media were betaine, choline, DL-homocysteine and L-methionine. They were purchased from Sigma.

S. cerevisiae strains, BY4742 (MATα his3Δ1 leu2-Δ0 lys2-Δ0 ura3-Δ0), BY4742Δmet6 (MATα his3Δ1 leu2-Δ0 lys2-Δ0 ura3-Δ0 met6-Δ::KAN) and BY4742Δmet15 (MATα his3Δ1 leu2-Δ0 lys2-Δ0 ura3-Δ0 met15-Δ::KAN) were purchased from EUROSCARF. The wild-type strain Σ1278b was from the Laboratory of Microbiology (Free University of Brussels, Brussels, Belgium). The Schizosaccharomyces pombe strain h+ ade6 was from the Institute of Microbiology (University of Wroclaw, Wroclaw, Poland). Yeast cells were grown on YPD medium [2% (w/v) glucose/2% (w/v) peptone/1% (w/v) yeast extract] or W0 medium [1% carbon source/0.57% yeast nitrogen base without amino acids] with 20 µg/ml of the supplements required for growth, at 30 °C.

The E. coli strains used were DH5α F’ for plasmid amplification and XL1 Blue (Stratagene) as a host strain for the A. nidulans λ Zap cDNA library.

Transformation of A. nidulans

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vector with a helper phage, according to the method prescribed by Stratagene.

**mRNA analysis**

The transcription start point was determined using 5’-full RACE (rapid amplification of cDNA ends) Core Set (Takara, Osaka, Japan) following the manufacturer’s method. The SM3LO primer was phosphorylated at the 5’-end with T4 kinase (Gibco BRL, Paisley, Renfrewshire, Scotland, U.K.) and it was used to amplify the 5’-terminal fragment of metH mRNA. Amplification of the PCR product containing the 5’-end of the SM3LO 5’-GTAACCGGTAAGACGCTCAC-3’ primer linked to the first nucleotide of the mRNA was achieved using the SM33UP 5’-TTCTCGTAGGCAGGCTTGAA-3’ and SM3LO 5’-TTCTCGTAGGCAGGCTTGAA-3’ primers. The resulting PCR fragments were used as a template for second amplification with the SM4UP 5’-AGTCTCTTTTCCACGTTCC-3’ and SM6LO 5’-TTCTTGAGGGAGCCGGTAGTA-3’ and SM7LO 5’-TTCTTGAGGGAGCCGGTAGTA-3’ primers. The final PCR fragment was sequenced on both strands.

The metH mRNA sequence in the intron–exon region was determined by sequencing the RT (reverse transcriptase)–PCR product obtained using total RNA, primers SM1UP and SM1LO and SuperScript™ One-Step RT–PCR System (Gibco BRL).

**Isolation of RNA**

Total RNA was isolated using TRI Reagent (Molecular Research Center, Cincinnati, OH, U.S.A.) according to the manufacturer’s instructions. For isolation of RNA from A. nidulans, mycelia were grown for 16 h at 37°C in 100 ml of liquid MM with supplements. Mycelia (200 mg) were immediately frozen in liquid nitrogen and ground in a mortar with powdered glass and 1.5 ml of TRIzol®. S. cerevisiae and Sch. pombe were grown in 100 ml of WO medium until the cultures reached A600 0.8. After centrifugation and washing with water, 2 ml of TRIzol® was added to the pellet and the cells were disrupted by vigorous vortex-mixing with glass beads. The RNA samples for 5’-transcript termini mapping and RT–PCR were dissolved in RNase-free water and treated with RNase-free DNaseI (Roche).

**Northern blotting**

Northern-blot analysis was performed as described in [30] with some modifications. The sample containing 20 µg of total RNA was glyoxylated [in 24 ml of buffer containing 15 mM sodium phosphate buffer (NaPi), pH 6.5/0.025% Bromophenol Blue/5% (v/v) glycerol/0.05% ethidium bromide/50% (v/v) DMSO/7% (w/v) glyoxal] at 55°C for 1 h followed by fractionation in 1% agarose in 15 mM NaPi (pH 6.5). Ethidium bromide-stained 28S rRNA was used to standardize the amounts of RNA loaded on to the gel. It was then capillary-transferred overnight to a nylon membrane (Hybond N⁺; Amersham Biosciences) in 25 mM NaPi (pH 6.5). The filters were prehybridized in 250 mM NaPi (pH 7.5) with 7% (w/v) SDS and 2% blocking reagent (Roche) for 3 h at 68°C. DNA probes were α-32P-labelled by random priming with the HexaLabel Plus DNA labelling kit (MBI Fermentas, Munich, Germany) and purified on SigmaSpin Post Reaction Purification Columns (Sigma). Hybridization was performed in the same buffer similar to prehybridization at 68°C overnight. Filters were washed with 20 mM Na2HPO4, 2 mM EDTA and 1% SDS for 10 min, five times at 65°C. Standard autoradiography was performed with a PhosphorImager system (Molecular Dynamics). The intensity of gene expression during induction or repression was estimated by quantification of the transcript level using the Image Quant system (Molecular Dynamics). In every case, at least three independent experiments and two independent experiments were performed.

For determining MS transcript levels, PCR fragments were synthesized with the following primers: for A. nidulans, SM3UP (see above) and SM4LO 5’-ACTTGCGTTTCGATGGA-3’; for S. cerevisiae, SCUP1 5’-CCTGCCGGCCATTAAGAGG-3’ and SCL01 5’-CCTGACGCAAGCTTGGA-3’; and for Sch. pombe, SPUP1 5’-CCTACCAAATGTTGATCC-3’ and SPL01 5’-GCAAGAGGTATAGCCGGTAA-3’.

**Extract preparation**

A. nidulans strains were cultured in 100 ml of MM with supplementation as indicated for 16–18 h at 37°C. Mycelia (500 mg) were then homogenized in a chilled mortar with powdered glass and 1–2 ml of 10 mM potassium phosphate buffer (KPi; pH 7.2). The extracts were centrifuged at 13000 g and supernatants were used for the enzyme assay. Protein concentration in the extracts was measured with Bio-Rad protein assay and adjusted to 2 mg/ml with the buffer.

**MS assay**

MS assay was described by Drummond et al. [31]. The reaction was linear with respect to time and concentration of protein, until the concentration of tetrahydrofolate reached 9.1 µM (A350 0.350). At this concentration tetrahydrofolate probably inhibits the enzyme activity. There were no differences in specific activities between crude extracts and extracts purified by filtration through Sephadex G-25.

**Betaine:homocysteine methyltransferase (BHMT) assay**

BHMT assay was performed as described by Skiba et al. [32]. In the reaction, 100 µg of total protein was used.

**Isolation of lipids and TLC**

A. nidulans strains were grown for 18 h in 50 ml of liquid MM containing 100 µM radiolabelled [glycine-1-14C]-betaine (50000 c.p.m./µmol; Moravek Biochemicals, Brea, CA, U.S.A.) or [methyl-14C]betaine (Biotrend Chemikalien, Cologne, Germany). Mycelia were ground in a mortar with powdered glass and 5 ml of the mixture chloroform/methanol/water (25:12.5:10, by vol.). Cell debris was removed by centrifugation; 1.25 ml of the mixture chloroform and 1.25 ml of 0.9% NaCl were added to the supernatant and the resulting phases were separated. The organic phase was dried. The residue containing lipids was dissolved in 500 µl of methanol. Lipid solution (100 µl) was applied on a Silica gel 60 F254 plate (Merck) and subjected to two-dimensional chromatography. The first development was in methanol/chloroform/ammonium hydrate (14:6:1, by vol.) and the second development was in chloroform/methanol/acetic acid/water (14:6:6:3, by vol.). Lipid spots were visualized using iodine vapour and identified by co-chromatography with standards. The radioactive metabolites were visualized by autoradiography.

**RESULTS**

**Cloning and analysis of metH gene**

The 5.1 kb XbaI–XhoI fragment of the W25A01 cosmids, which restored the met+ phenotype in the metH2 mutant was cloned...
into the pUC19 vector giving the pLX2 plasmid. The sequence of 4323 bp from this fragment was submitted to the GenBank® under the accession no. AF275676. It contains an open reading frame encoding a protein of 774 amino acids showing 61% identity with S. cerevisiae cobalamin-independent MS and 47% with both A. thaliana and E. coli enzymes. As revealed by comparison with the cDNA copy, the open reading frame is interrupted by one 65 bp intron, beginning at 16th nucleotide downstream from the putative ATG initiation codon. The fragment also comprises 1123 nt upstream from the coding sequence and a 720 nt downstream from the stop codon.

This gene was also sequenced in the metH2 and metH10 strains, and was found to be mutated in both of them: metH2 has a tyrosine to cysteine transition causing a Y635 → H substitution, whereas metH10 has a mutation located at the 5'-splice site of the intron; the wild-type sequence C/GTATGC is changed in the third position to C/GTATGC which may impair the splicing process. These findings confirmed that the cloned MS gene was metH and not a suppressor of the metH2 mutation.

Since both the metH2 and metH10 strains are leaky and show an appreciable MS activity (see below) we attempted to determine whether metH is an essential gene. The gene was disrupted and the resulting strain was a tight auxotroph. It was viable on methionine showing that metH is not an essential gene.

The S. cerevisiae Δmet6 mutant, lacking MS, grew on galactose-supplemented medium when transformed with a plasmid carrying metH cDNA under the yeast GAL1 promoter form pYES2 vector (Stratagene). This indicated that the A. nidulans enzyme is functional in S. cerevisiae.

**Homocysteine increases MS mRNA levels**

The nearest transcription start point of the metH gene is positioned at −174 nt from the predicted ATG-initiation codon as determined by 5'-RACE analysis (see the Experimental section). The regulation of gene transcription under various growth conditions was studied by Northern-blot analysis. It was found that the transcript level was affected neither by concentration of sulphate (0.1–2.0 mM) and methionine (0.25–5.0 mM) nor by the regulatory mutations sconB2 and metR1 (results not shown).

On the other hand, the transcription was strongly increased in cells grown in the presence of homocysteine (Figure 2A), even at a concentration as low as 0.25 mM. At least 400% increase in the transcript level was repeatedly observed in experiments with 3 mM homocysteine. The inducing effect of homocysteine was confirmed in the mutants impaired in sulphur metabolism (Figure 2B). As compared with the wild-type, a lower level (by at least 50% of the transcript level) was observed in the metG55 strain, lacking cystathionine β-lyase and accumulating cystathionine, whereas it was higher by approx. 4-fold in the mecA1 strain impaired in cystathionine β-synthase, which should be expected to have a higher level of homocysteine.

It is of interest to determine whether the transcriptional regulation of MS-encoding genes by homocysteine is a more general phenomenon, at least in fungi. As shown in Figure 2(C), homocysteine in the growth medium stimulated transcription of the MS genes both in S. cerevisiae and Sch. pombe.

A slight increase (from 140 to 160%) in MS transcript level was observed in the mecC13 mutant partially blocked in SAM synthase (Figure 3) leading to reduction of cellular SAM to 30 % of the wild-type [33]. This suggested that a shortage of SAM stimulates metH expression.

A very low transcript level was found in metH10 (Figure 4). RT–PCR fragments obtained with RNA isolated from the mutant had a sequence identical with those obtained for RNA isolated from the wild-type. This showed that the intron was spliced correctly but with a low efficiency. As a consequence, metH10 makes a normal enzyme but in a low amount which is responsible for the met− phenotype. Unspliced or wrongly spliced mRNA was probably degraded. Its level in the mutant, even in the presence of homocysteine in the growth medium never exceeded 60% of the control. In the metH2 mutant grown on a medium containing
Regulation of methionine synthase from Aspergillus nidulans

Figure 4  Expression of metH gene in metH mutants

Mycelia were grown on MM with: 1, 5 mM methionine; 2, 0.25 mM methionine and 2 mM homocysteine. The amounts of metH transcript level relative to its content in the wild-type strain grown on MM with 0.25 mM methionine are shown at the bottom (means from two experiments). Percentage increases in transcript levels have been corrected for variations in the levels of 28 S RNA.

low methionine concentration, the transcript level was 1.5-fold higher than in the wild-type, probably due to the accumulation of homocysteine. This was not observed at high concentrations of methionine since sulphate assimilation and homocysteine synthesis are repressed in this condition.

Homocysteine stimulates MS expression at the protein activity level

MS activity was assayed in the wild-type strain and several mutants grown under various conditions of sulphur supplementation. The results shown in Figure 5 indicate that, in contrast with its effect on transcription, methionine has some suppressing effect on the enzyme level whereas homocysteine enhances it more than 3-fold. A similar effect of homocysteine was observed in metH2, which showed approx. 50% of the wild-type activity (surprisingly high, by taking into account the slight leakiness of the mutant). There were also no significant differences between the Km values for MS in these strains: 60.4 ± 6.5 and 87.7 ± 10.3 µM for CH3H4PteGlu3 and 6.2 ± 0.9 and 6.7 ± 1.6 µM for homocysteine for the wild-type and metH2 strains respectively.

Regarding other mutant strains affected by sulphur metabolism, an increased enzyme activity was found in mecA1, which corresponded to the increased transcript level observed in this strain. However, the metG55 strain showed MS activity similar to that of the wild-type despite a lower transcript level. In contrast, the high transcript level in the mecC13 mutant did not coincide with an increase in enzyme activity.

No MS activity was found in the strain containing a disrupted metH gene. This indicated that the enzyme activity observed in the metH mutants could not be attributed to an isoenzyme encoded by another MS gene.

Betaine negatively regulates the expression of MS on mRNA and protein levels

As mentioned earlier, betaine stimulates growth of metH10. This raised the question: what is the mechanism by which this compound stimulates growth of this strain? It does so in spite of the fact that it strongly represses metH transcription (Figure 6) and enzyme activity (Figure 5). Betaine (1 mM) always led to >80% repression of metH transcript level. Betaine is known
to be a donor of a methyl group for methylation of homocysteine in a reaction catalysed by BHMT. Such an enzyme is known to be expressed in mammalian liver and kidney cortex [34] and BHMT activity has also been reported in halophytic bacteria [35,36]. We looked for this activity in A. nidulans mycelial extracts but the results were negative (a previous report of BHMT activity in A. nidulans [42] was incorrect).

Experiments using choline-supplemented media and choline-requiring mutant choA1 (Figure 7) have shed light on the role of betaine. First, choline could substitute for betaine in stimulating the growth of metH10. This was not surprising since choline is known to be a precursor of betaine. An unexpected finding, however, was the growth of the choA1 strain on betaine-supplemented medium. This suggested that betaine can be converted into choline.

To verify this possibility, the metH10 strain was grown in the presence of[^14]CH_3]betaine. The lipids were then extracted from the mycelium and separated by TLC. The lipid fraction contained approx. 40% of the total incorporated radioactivity and practically all of that was found in one radioactive spot corresponding to phosphatidylcholine (Figure 8). Identical results were obtained with betaine labelled in the glycine backbone (results not shown), indicating that betaine does not serve directly or indirectly as a methyl donor for ethanolamine methylation.

Therefore it appears that the observed effects of betaine on growth and transcription are, in fact, brought about by choline made from it or its derivative. As shown in Figures 5 and 6, supplementing the growth medium with choline leads to a decrease in both the transcript and enzyme levels.

**DISCUSSION**

The present study describes a novel type of regulation of the MS in A. nidulans: the enzyme synthesis is positively regulated by homocysteine and negatively regulated by betaine, both at the transcriptional level. Both mRNA and active enzyme levels were increased by supplementation with homocysteine. Supplementing with choline resulted in repression of metH10 transcription and lower levels of MS enzyme. In contrast with MS genes from other organisms, the gene was not regulated by methionine. Also no regulation by the cysteine-mediated SMR system was found.

The regulation of MS gene expression by homocysteine, already observed in S. typhimurium and E. coli [3,4], may be also common in fungi, since we have observed it not only in A. nidulans but also in two yeast species S. cerevisiae and Sch. pombe. It appears that this regulation of metH transcription is subtle, as demonstrated by the different transcript levels in mutant strains depending on whether they accumulate the amino acid or experience a shortage of it. This precise regulation may also result from a need to remove homocysteine, which is toxic at higher concentrations.

Homocysteine is known to be toxic to A. nidulans [37] and S. cerevisiae [3,38] mutants impaired in cystathionine β-synthase and therefore unable to metabolize it to cysteine (Figure 1). This toxicity was attributed to the conversion of homocysteine to homocysteine thiolactone, which unproductively consumes considerable amounts of ATP [38]. This conversion is performed by methionyl-tRNA synthase, which misactivates homocysteine and then edits it by cyclization of homocysteine adenylate [38]. In addition, homocysteine thiolactone, being a chemically reactive compound, forms adducts with the ε-amino group of protein lysine residues, increasing the detrimental effect of homocysteine on cell growth [39].

It is possible that, for the same reasons, the addition of homocysteine is toxic for the metH2 and metH10 strains. Neither metabolize homocysteine effectively owing to decreased levels of MS activity and therefore contain relatively high homocysteine and low methionine levels. Lower activities of the enzyme in the mutants are caused by different mechanisms. The metH10 strain produces low levels of wild-type enzyme, even after homocysteine supplementation, owing to a reduced amount of MS transcript. The metH2 strain makes a normal or even increased amount of the transcript, so it is expected to produce a normal amount
of less-active enzyme. The MS activity in the mutant grown in the presence of homocysteine is 2-fold lower when compared with that in the wild-type strain grown under the same conditions.

SAM starvation led to an increase in metH transcription, but no induction was observed in the enzyme level in contrast with the observation made by Burton and Metzenberg [15] in N. crassa. In accordance with the results obtained with N. crassa, repression of the enzyme activity was brought about by betaine. Betaine exerted the same effect and both compounds repressed the metH gene transcription.

Experiments with A. nidulans strains grown in the presence of radiolabelled betaine showed that a large proportion of the radioactivity incorporated into the mycelium was found in phosphatidylcholine. Identical results were obtained with 14C-betaine labelled on the methyl group and on the glycine backbone. The straightforward interpretation of these results is that betaine is reduced to choline. Conversion of betaine to choline had been commonly believed to be an irreversible process, but recent observations in Penicillium fellutanum made by Park et al. [22] strongly suggested such a possibility. Intermediates in this pathway remain to be determined. Park et al. [22] suggested that the carboxy group of betaine may be phosphorylated and then reduced to betaine aldehyde, as it is for the L-threonine biosynthetic pathway, where aspartate is phosphorylated to aspartyl-β-phosphate and subsequently reduced to aspartyl-β-semialdehyde. Another possibility is that some multi-specific dehydrogenase may perform the reduction of betaine to betaine aldehyde since such a reduction has been demonstrated in vitro by glyceraldehyde dehydrogenase (EC 1.2.1.21) in E. coli [40].

The homocysteine-dependent induction and choline (betaine)-dependent repression of MS gene transcription represents a novel type of regulation in the sulphur metabolic network in A. nidulans besides the SMR and methionine-mediated systems. It remains to be determined if such a complex regulation of MS genes also takes place in other organisms. The repression by choline is not surprising since MS can be regarded not only as the last enzyme in methionine synthesis but also as the first in choline synthesis. The latter process consumes a major fraction of the methyl groups of methionine. It was found that, in plants, 46% of MS activity contributes to the synthesis of phosphatidylcholine [41]. Thus, when choline is supplied, the demand for methyl groups, and therefore for methionine, is reduced. In such a situation, choline exerts feedback inhibition on metH transcription. This explanation is supported by the fact that only leaky metH mutants, including partial revertants of the metH2 strain (results not shown), responded to betaine and choline. These compounds are ineffective when the synthesis of methionine falls below the threshold needed for protein synthesis, since they cannot replace the amino acid in this role.

It is worth noting that there is no strict correlation between transcript level and the enzyme activity. The presence of methionine in the growth medium, which does not repress the transcript level, decreases the enzyme-specific activity by approx. 20%. Moreover, the 5-fold induction of transcription by homocysteine leads only to a 3-fold induction of activity. Similarly, more than 3-fold repression of transcript level by betaine causes only a 2.5-fold decrease in the enzyme level. A mutant with impaired homocysteine synthesis (metG55), in which metH transcript level is low, shows a wild-type level of MS, whereas the increased homocysteine level in the mecC13 mutant, causing transcription induction, also increases enzyme activity. An increased activity of the enzyme was also observed in the sconB mutant despite the fact that the transcript level did not differ from that in the wild-type. On the other hand, the increased transcript level in the mecC13 mutant, caused not by homocysteine but by SAM deficit, did not lead to increased MS-specific activity. These results strongly suggest that the synthesis of MS in A. nidulans is also regulated post-transcriptionally.

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