In vivo hydrolysis of S-adenosylmethionine induces the *met* regulon of *Escherichia coli*

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Regulation of methionine biosynthesis in *Escherichia coli* involves a complex of the MetJ aporepressor protein and S-adenosylmethionine (SAM) repressing expression of most genes in the *met* regulon. To test the role of SAM in the regulation of *met* genes directly, SAM pools were depleted by the *in vivo* expression of the cloned plasmid vector-based coliphage T3 SAM hydrolase (SAMase) gene. Cultures with *in vivo* SAMase activity were assayed for expression of the *metA*, *B*, *C*, *E*, *F*, *H*, *J*, *K* and *R* genes in cells grown in methionine-rich complete media as well as in defined media with and without L-methionine. *In vivo* SAMase activity dramatically induced expression between 11- and nearly 1000-fold depending on the gene assayed for all but *metJ* and *metH*, and these genes were induced over twofold. *metJ*::Tn5 (aporepressor defective) and *metK*::Tn5 (SAM synthetase impaired; produces <5% of wild-type SAM) strains containing *in vivo* SAMase activity produced even higher *met* gene activity than that seen in comparably prepared cells with wild-type genes for all but *metJ* in a MetJ-deficient background. The SAMase-mediated hyperinduction of *metH* in wild-type cells and of the *met* genes assayed in *metJ*::Tn5 and *metK*::Tn5 cells provokes questions about how other elements such as the MetR activator protein or factors beyond the *met* regulon itself might be involved in the regulation of genes responsible for methionine biosynthesis.

Received 4 September 2005

Revised 25 January 2006

Accepted 27 January 2006

INTRODUCTION

Methionine biosynthesis requires enzymes encoded by seven widely scattered genes of the *met* regulon divided between two short convergent pathways (Fig. 1; reviewed by Greene, 1996). The combined activities of MetA, MetB and MetC replace the hydroxyl group of L-homoserine with a thiol moiety to form L-homocysteine. Simultaneously, GlyA and MetF transfer and reduce one carbon from serine to tetrahydrofolate (THF), producing N⁵-methyl-THF. Either of two methyltransferases, the products of the *metE* and *metH* genes, transfers the methyl group from THF to homocysteine to form methionine. Methionine then either participates in protein synthesis or is condensed by the *metK*-encoded SAM synthetase with ATP to produce the ubiquitous nucleotide S-adenosyl-L-methionine (SAM).

Mutating either of two genes produces constitutive expression of most genes in the *met* regulon and excess production and even export of methionine and its metabolites (Usuda & Kurahashi, 2005). Mutants of the more common type have decreased *in vivo* pools of SAM as a result of impaired SAM

synthetase activity, and the defects map to *metK* (Greene *et al.*, 1973; Hafner *et al.*, 1977). Mutations of the second class map to *metJ*, and genetic and biochemical evidence identified the corresponding small protein as a DNA-binding aporepressor of *met* gene transcription (Su & Greene, 1971). These results suggested that a complex of MetJ and SAM binds a common nucleotide sequence in the promoters of target genes to prevent their transcription (Greene *et al.*, 1970, 1973; Hobson & Smith, 1973). Identification of a consensus repressor binding site ('met box'; Belfaiza *et al.*, 1986) and crystallographic studies (Rafferty *et al.*, 1989) all support this model. Genes most induced by defects in either *metJ* or *metK* include *metA*, *B*, *C*, *E*, *F*, *K*, *L* (as the downstream gene in the *metBL* operon) and *metR* (a regulatory protein that, by itself or when bound with homocysteine, regulates expression of a variety of genes in the *met* regulon to coordinate the activities of the two branches of the methionine biosynthetic pathway; again, see Greene, 1996). Cells thus regulate methionine biosynthesis through a feedback mechanism that monitors levels of SAM to activate *met* gene expression before the concentration of methionine decreases to levels that could impair protein synthesis. In addition to the MetJ–SAM repressor complex, other regulatory mechanisms, including the effects of the activator MetR protein (Urbanowski *et al.*, 1987) and regulation of *metA* expression by heat shock (Biran *et al.*, 1995) and of MetA stability by proteolysis (Biran *et al.*,

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Abbreviations: SAM, S-adenosyl-L-methionine; SAMase, SAM hydrolase.

Fig. 1. Biosynthesis of methionine and SAM in *E. coli*. Methionine is produced as the result of a two-branch pathway (see Greene, 1996). In one branch, the hydroxyl group of homoserine is converted to the thiol group of homocysteine by the sequential actions of the products of the *metA*, *metB*, and *metC* genes. In the other branch, a methylene group is transferred from serine to tetrahydrofolate (THF) and then reduced to form 5-methyl-THF by the products of the *glyA* and *metF* genes. The methyl group is then transferred from the THF by the products of either the *metE* or *methH* genes to the thiol group of homocysteine to produce methionine. Finally, methionine is condensed with ATP to produce SAM by SAM synthetase, the product of *metK*. According to the current model, the resulting SAM then binds to the MetJ aporepressor to repress expression of *metA*, *B*, *C*, *E*, *F*, *K*, *R* and *glyA*; only *metJ* and *methH* are not known to be repressed directly by the MetJ–SAM complex.

2000), make regulation of elements of the *met* regulon highly complex.

Though well supported by genetic and biochemical data, the role of SAM has not been directly tested *in vivo*. This is due to three problems inherent in studies involving SAM in bacteria: SAM typically cannot cross bacterial cell membranes (including those of *Escherichia coli*), SAM synthetase inhibitors (e.g. methionine analogues such as ethionine) induce side effects unrelated to SAM deprivation (Alix, 1982; Pine, 1978), and known viable SAM synthetase mutants are leaky even under restrictive conditions (Hafner *et al.*, 1977; Mulligan *et al.*, 1982). We circumvented these difficulties by transforming cells with plasmid expression vectors containing the cloned coliphage T3 SAM hydrolase (SAMase) gene, resulting in *in vivo* SAMase activity not otherwise found in *E. coli*. This enzyme cleaves SAM into homoserine and 5'-methylthioadenosine, removes SAM from the cell, and inhibits a variety of SAM-related activities (Hughes *et al.*, 1987; Posnick & Samson, 1999; Val & Cronan, 1998). Introducing SAMase activity should specifically remove the co-repressor without affecting the MetJ aporepressor and

demonstrate the effect of reduced SAM concentrations on the expression of elements of the *met* regulon. Results from our assays of *met* gene expression in cells containing *in vivo* SAMase activity firmly support the role of SAM as the co-repressor. They also indicate that other factors – including and possibly in addition to MetR – play roles in influencing SAM-related *met* gene regulation.

METHODS

Bacterial strains and growth conditions. All strains used were derived from the *E. coli* K-12 strain BW545 and are listed in Table 1; the YT complete and M9 defined media used are those of Miller (1972). Assays of enzyme activities other than the expression of *met::lacZ* fusions were performed on BW545. As described by Mulligan *et al.* (1982), the GW strains were identified by screening for defects in methionine metabolism after *Mud::Ap,lac*-mediated insertional mutagenesis of BW545, and the *metJ::Tn5* and *metK::Tn5* markers were inserted into the originally identified and stabilized *met* reporter strains by P1_{vir} transduction. BWmJ and BWmK are *metJ::Tn5* and *metK::Tn5* derivatives of BW545 produced by P1_{vir} transduction (Mares *et al.*, 1992) of the respective markers from GW2529 and GW2533. They were identified by screening for resistance to 50 µg kanamycin ml⁻¹ on YT agar plates after P1_{vir} transduction and subsequent demonstration of elevated MetC activity using the assay described below. *E. coli* BW545, BWmJ (*metJ::Tn5*) and BWmK (*metK::Tn5*) lysogenic for bacteriophage λgt2 constructs containing *Salmonella typhimurium met* gene promoter::*lacZ* fusions were assayed for *metB* (λBlac; Urbanowski & Stauffer, 1986), *metE* (λElac; Plamann *et al.*, 1988), *metF* (λFlac; Stauffer & Stauffer, 1988), *metH* (λHlac; Urbanowski & Stauffer, 1989b), *metJ* (λJlac; Urbanowski & Stauffer, 1986) and *metR* (λRlac; Urbanowski & Stauffer, 1987) expression. In all cases, cells used to inoculate liquid cultures came from colonies raised overnight on agar plates inoculated with either freshly transformed cells or transformants stored in 15% (v/v) glycerol at -70 °C and streaked for purification. Prior to assay, cultures were grown overnight with shaking at 32 °C in test tubes containing 3 ml liquid medium containing 100 µg ampicillin ml⁻¹ and/or 15 µg tetracycline ml⁻¹.

Plasmids and transformations. Plasmids were constructed, stored, and transformed into bacteria according to Maniatis *et al.* (1982). The SAMase gene cloned into M13mp8 that produced M13hb1 (Hughes *et al.*, 1987) was removed by *EcoRI* and *HindIII* digestion and ligated into pUC18 (Yanisch-Perron *et al.*, 1985) to

Table 1. *E. coli* K-12 strains used

Strain descriptions and storage conditions are given in Methods, and strains from G. Walker are described in Mulligan *et al.* (1982).

Strain	Genotype	Source
BW545	Δ <i>lacU169 rpsL</i>	G. Walker
BWmJ	BW545, <i>metJ::Tn5</i>	This work
BWmK	BW545, <i>metK::Tn5</i>	This work
GW2517	BW545, Φ(<i>metA'-lacZ</i> ⁺)-168, Ap ^r	G. Walker
GW2521	BW545, Φ(<i>metF'-lacZ</i> ⁺)-170	G. Walker
GW2522	BW545, Φ(<i>metE'-lacZ</i> ⁺)-171	G. Walker
GW2529	GW2517, <i>metJ::Tn5</i>	G. Walker
GW2533	GW2517, <i>metK::Tn5</i>	G. Walker

produce pHBF1UC. Because we questioned the ability of ampicillin to enforce long-term maintenance of this plasmid, pHBF1UC was cut with *Pst*I and ligated to *Pst*I-cut pBR322 (Bolivar *et al.*, 1977) to form pHBBR2 and gain the tetracycline resistance that ensured plasmid maintenance. Also, the promoterless *Eco*RI–*Bam*HI SAMase gene removed from M13hb1 was inserted into pBR322 to produce pHBF1BR, a stably propagated plasmid under ampicillin selection that directs weak SAMase expression, apparently from a cryptic promoter.

Enzyme assays. Cystathionine synthetase (MetB) activity was determined in toluenized cells by the *O*-succinylhomoserine-dependent oxidation of reduced nicotinamide adenine dinucleotide, measured as A_{340} (Holloway *et al.*, 1970; Kaplan & Flavin, 1966). Cystathionase (MetC) activity was assayed by the cystathionine-dependent reduction of Ellman's reagent, measured as A_{410} (Flavin, 1962; Holloway *et al.*, 1970). β -Galactosidase expression of *met::lacZ* fusion genes was monitored by the rate of ONPG hydrolysis (Miller, 1972). SAMase was assayed by passing reaction mixes inoculated with $^{14}\text{COOH-SAM}$ (Amersham Biosciences) over Affi-gel 601 (Bio-Rad) cis-diol affinity columns and determining the net percentage of total counts that failed to bind the column and were assumed to be in $^{14}\text{COOH-L-homoserine}$ (Hughes *et al.*, 1987). SAM synthetase activity was measured as the percentage of [^{35}S]methionine (Amersham Biosciences) converted to labelled cis-diol (Affi-gel 601)-binding compounds (e.g. SAM and its metabolites *S*-adenosylhomocysteine, 5'-methylthioadenosine and 5'-methylthioribose) after incubation with toluenized cells under conditions for the SAM synthetase assay (Hafner *et al.*, 1977; Hughes *et al.*, 1987). Protein concentrations were determined by the Lowry method. In all cases, assays were conducted on at least five cultures from independently transformed cells, grown to stationary phase, performed according to minor modifications of published procedures, and quantified spectrophotometrically (with a Spectronic 21 or Beckman DU-64) or by liquid scintillation (Beckman LS5000TD) using Beckman ReadySafe scintillation cocktail. Statistical comparisons employed Student's *t* test, with $P < 0.05$ indicating significant differences between paired samples.

RESULTS

Evidence of SAMase expression

Results throughout this study reflect the effect of *in vivo* SAMase expression directed either by pHBF1BR, a vector that results in the synthesis of a low level of SAMase activity ($0.13 \text{ nmol min}^{-1} \text{ mg}^{-1}$ in whole-cell extracts), and pHBBR2, a vector that produces a higher level of SAMase expression ($0.97 \text{ nmol min}^{-1} \text{ mg}^{-1}$ in whole-cell extracts). Cells with these vectors were compared against cells transformed with pBR322, in which SAMase activity was not detected. While cells containing pHBF1BR showed no obvious differences in growth rate or morphology when compared with negative controls, those with pHBBR2 grew more slowly (and with an especially pronounced and highly variable lag period), and individual cells often showed extreme filamentation, all as reported previously (Hughes *et al.*, 1987).

Effect of *in vivo* SAMase expression in *met* wild-type cells in a complete medium

Three systems were used initially to survey the consequence on *met* gene expression of the *in vivo* expression of SAMase in *E. coli* K-12 BW545. The results of these assays are presented in Table 2.

Cystathionine synthetase (*metB*), cystathionase (*metC*) and SAM synthetase (*metK*) expression is repressed in the presence of methionine and wild-type *metJ* and *metK* gene products. We first monitored expression of these enzyme activities directly in cells with *in vivo* SAMase activity. This intracellular activity evoked substantial induction of MetB, C and K activities, suggesting that *in vivo* hydrolysis of SAM impaired the ability of the cells to repress these elements of the *met* regulon.

Secondly, we expressed SAMase in the BW545-derived GW strains that carry the *lacZ* gene fused behind the host's promoters for *metA*, *metE* and *metF*. The use of these *met-lac* fusion strains simplified the assays and expanded our survey to include another three genes. Introduction of SAMase activity into GW2517 (*metA::lacZ*), GW2521 (*metF::lacZ*) and GW2522 (*metE::lacZ*) also greatly enhanced expression of these genes.

We then lysogenized BW545 with recombinant λ gt2 phage carrying *metB*, *E*, *F*, *H*, *J* or *R* gene promoters from *S. typhimurium* fused to *lacZ*, constructs that have been used extensively to assay *met* gene expression in *E. coli* (see Methods for appropriate references). *metB*, *E*, *F* and *R* showed dramatic induction of activity while *metJ* and *metH* showed much smaller but still significant increases. Both the *E. coli* and *S. typhimurium* promoters for *metE* and *metF* responded similarly to the introduction of *in vivo* SAMase activity, although the *S. typhimurium* promoters directed a somewhat higher basal level of gene expression. While results from the cystathionine synthetase (MetB) assays showed less enhanced enzyme expression than those revealed by assays of MetB activity gauged through the *S. typhimurium* promoter *met-lac* fusions, in both cases the assays indicated substantial SAMase-mediated enzyme induction.

Because of the similarity of results seen using *E. coli* and *S. typhimurium* promoters, the simplicity of the reporter assays, and the fact that BW545 λ lysogens are *met* wild-type strains while GW strains are methionine auxotrophs, we used these lysogens for all subsequent experiments. We deemed the use of *met* wild-type cells to be especially important. For example, defects in homocysteine biosynthesis in MetA[−] strains or in its use in MetE[−] or MetF[−] strains would likely alter MetR-mediated gene activation (Byerly *et al.*, 1990; Cowen *et al.*, 1993; Mares *et al.*, 1992; Urbanowski *et al.*, 1987) and introduce complex and unpredictable effects beyond those provoked by SAMase expression.

Effect of *in vivo* SAMase expression in *met* wild-type cells in a methionine-limited medium

Because cells transformed with pHBBR2 grow poorly in defined media (data not shown), we routinely cultured cells in antibiotic-supplemented YT complete medium. On the other hand, it was necessary to grow cells in a methionine-deficient medium to determine the impact of methionine on *met* gene expression. To ensure reliable growth, cells were first cultured overnight in YT medium,

Table 2. Effect of *in vivo* SAMase activity on *met* gene expression in *met* wild-type *E. coli*, comparing the effects of different genes and promoters from *E. coli* and *S. typhimurium*

All cultures were inoculated from independent colonies of freshly transformed cells and incubated overnight in YT medium containing 15 µg tetracycline ml⁻¹ at 32 °C with shaking. Numbers represent means of values from at least five cultures for each condition. ND, Not determined.

<i>met</i> gene	Plasmid used	Native enzyme assays*		<i>met-lac</i> fusion assays‡			
		Activity	<i>met</i> induction†	<i>E. coli met</i> promoter		<i>S. typhimurium met</i> promoter	
				Activity	<i>met</i> induction	Activity	<i>met</i> induction
A	pBR322	ND	—	4·21	384	ND	—
	pHBBR2	ND		1620		ND	
B	pBR322	6·78	12·2	ND	—	41·6	27·2
	pHBBR2	83·1		ND		1130	
C	pBR322	13·0	11·4	ND	—	ND	—
	pHBBR2	148		ND		ND	
E	pBR322	ND	—	0·852	458	32·9	34·0
	pHBBR2	ND		390		1120	
F	pBR322	ND	—	2·06	930	22·4	62·0
	pHBBR2	ND		1920		1390	
K	pBR322	0·180	24·4	ND	—	ND	—
	pHBBR2	4·39		ND		ND	

*MetB native enzyme activities are reported by the *O*-succinylhomoserine-dependent oxidation of reduced nicotinamide adenine dinucleotide, measured as A_{340} (Holloway *et al.*, 1970; Kaplan & Flavin, 1966). MetC native enzyme activities are reported as the cystathionine-dependent reduction of Ellman's reagent (Flavin, 1962; Holloway *et al.*, 1970). MetK native enzyme activities are reported by incorporation of [³⁵S]methionine into cis-diol-containing compounds (Hafner *et al.*, 1977; Hughes *et al.*, 1987).

†‘*met* induction’ represents the ratio of enzyme activity in cells transformed with pHBBR2 (SAMase expression vector) to cells transformed with pBR322, a negative control. All resulting ratios represent a significant induction of *met* gene activity according to Student's *t* test ($P < 0.05$).

‡MetA, B, E and F expression values are reported by chromosomally inserted *met::lacZ* fusion constructs and quantified by the enzymic hydrolysis of ONPG in β -galactosidase units (Miller, 1972).

washed and diluted 10-fold into YT medium, M9 minimal medium or M9 medium supplemented with L-methionine, and then cultured once again overnight. This regimen allowed growth in defined media and showed a reliable pattern of methionine-influenced gene expression (Table 3). With the exception of *metH* and *metJ*, the tested genes were significantly induced in cells grown without methionine in the absence of SAMase, while *in vivo* SAMase activity provoked a significant induction of expression for every gene tested under all conditions. The *metA*, *B*, *E*, *F* and *R* genes were most dramatically affected by either the absence of methionine from the culture medium or the presence of the SAMase expression vector. *metC* expression showed less dramatic results, although it is possible the different and possibly less sensitive method used to assay *metC* activity accounted in part for its apparently lower degree of sensitivity to either condition.

Effect of varying levels of SAMase activity on *met* gene expression

Table 4 illustrates the results derived from *met::lacZ* fusion strains expressing low or high levels of SAMase upon their

transformation with pHBF1BR or pHBBR2, respectively. In all cases, genes that showed dramatic induction in cells containing high levels of *in vivo* SAMase activity (*metB*, *metE*, *metF*, and *metR* from Table 2) were induced to a lesser degree in cells expressing the nearly 10-fold lower levels of SAMase activity resulting from the introduction of pHBF1BR. Expression of *metH* and *metJ*, genes only weakly induced by pHBBR2-directed SAMase activity, was not significantly induced in cells expressing the lower levels of SAMase directed by transformation with pHBF1BR.

Effect of *in vivo* SAMase expression in *metJ::Tn5* and *metK::Tn5* cells

Cells containing *metJ* or *metK* alleles that block or reduce expression of active proteins express *met* genes constitutively due to either the lack of the MetJ aporepressor or insufficient SAM to bind MetJ and repress the genes, respectively. We transformed previously reported *metJ::Tn5* (MetJ defective) and *metK::Tn5* (SAM synthetase impaired; a leaky construct that allows viability but greatly reduced synthesis of SAM) mutants produced in BW545 (Mulligan *et al.*, 1982) with pHBBR2 to determine whether

Table 3. Effect of *in vivo* SAMase expression in *met* wild-type *E. coli* in a methionine-limited medium

Cells were incubated overnight in YT medium containing 15 µg tetracycline ml⁻¹ at 32 °C with shaking, washed, resuspended in 10 × volume of the media indicated in the table (each containing 15 µg tetracycline ml⁻¹) and incubated again overnight under the same conditions prior to enzyme assay. Numbers represent means of values from at least five cultures for each condition.

<i>met</i> gene	Plasmid used	YT medium*		M9L-met medium*		M9 medium*	
		<i>met</i> activity†	<i>met</i> induction†	<i>met</i> activity†	<i>met</i> induction†	<i>met</i> activity†	<i>met</i> induction†
A‡	pBR322	10.6	109	36.5	36.7	453	3.88
	pHBBR2	1160		1340		1760	
B	pBR322	18.7	59.9	37.1	47.4	181	8.12
	pHBBR2	1120		1760		1470	
C§	pBR322	5.32	6.44	9.88	7.03	23.0	3.10
	pHBBR2	34.3		69.5		71.4	
E	pBR322	24.7	110	32.4	58.3	165	13.9
	pHBBR2	2720		1890		2290	
F	pBR322	14.5	157	95.5	24.3	476	6.62
	pHBBR2	2280		2330		3150	
H	pBR322	42.7	1.44	59.8	1.38	50.8	1.97
	pHBBR2	61.7		82.3		100	
J	pBR322	41.4	3.2	36.3	3.80	53.2	3.12
	pHBBR2	133		139		166	
R	pBR322	36.3	26.4	124	9.68	267	3.66
	pHBBR2	968		1200		978	

*YT (yeast tryptone), M9 and M9L-met (M9 containing 40 µg L-methionine ml⁻¹) are described by Miller (1972).

†‘*met* activity’ represents the expression of reporter-directed β-galactosidase assayed according to Miller (1972); ‘*met* induction’ is the ratio of enzyme activity in cells with *in vivo* SAMase activity (transformed with pHBBR2) to cells lacking *in vivo* SAMase activity (containing pBR322). Except as noted below, all ratios show significant induction of SAMase-mediated *met* gene activity (Student’s *t* test, *P* < 0.05).

‡While all expression values reported by chromosomally inserted *met::lacZ* fusion constructs were quantified by the enzymic hydrolysis of ONPG in β-galactosidase units (Miller, 1972), those for *metA* were measured from *E. coli* promoters of GW2517 while all others used the λgt2-based *S. typhimurium met* promoters in BW545 lysogens.

§MetC native enzyme activities are reported as the cystathionine-dependent reduction of Ellman’s reagent measured as A₄₁₀ (Flavin, 1962; Holloway *et al.*, 1970).

||Not significantly induced (Student’s *t* test, *P* > 0.05).

in vivo SAMase expression in *met* gene derepressed cells would have a significant effect on *met* gene expression. Table 5 shows the results of these assays. Assays on *metJ::Tn5* cells revealed an approximately 1.5–4-fold increase in the activity of five of the six tested *met* genes in the presence of *in vivo* SAMase activity; *metJ* itself is the exception. Assays of *met* gene expression in the *metK::Tn5* background showed over 10-fold induction for *metB*, *metE*, *metF* and *metR*; the increases in *metH* expression resembled the lower levels of induction seen in all of the genes assayed in the Met⁻ cells, and *metJ* expression in this case was significantly enhanced.

DISCUSSION

The dramatic induction of *metA*, *B*, *C*, *E*, *F*, *K* and *R* upon expression of *in vivo* SAMase activity in *E. coli* supports the

model of a MetJ–SAM repressor complex binding *met* gene promoters to block their transcription. SAMase expression presumably decreases *in vivo* pools of SAM, removing the corepressor and preventing formation of the complex that normally represses these target genes. On the other hand, SAMase activity has a lesser effect on the two genes in this group not known to be regulated (at least directly) by the MetJ–SAM repressor complex, *metH* and *metJ*. Because cells were grown in a complete medium containing levels of methionine sufficient to prevent induction of the genes in control cells, these results provide positive evidence that, as expected from previous reports, SAM and not methionine or some other methionine-related metabolite is the active second element of the repressor complex. These data also complement previously published findings on the pattern of *met* gene expression in cells grown in methionine-deficient (hence, SAM-limited) media (Table 3; Pine, 1978);

Table 4. Effect of varying levels of SAMase expression on *met* gene expression

All cultures were inoculated from independent colonies of freshly transformed cells and incubated overnight in YT medium containing 15 µg tetracycline ml⁻¹ at 32 °C with shaking. Numbers represent means of values from at least five cultures for each condition.

<i>met</i> gene*	Plasmid used	<i>met</i> activity	<i>met</i> induction ratio†	
			pHBF1BR/pBR322	pHBBR2/pBR322
<i>B</i>	pBR322	41·6	1·80	27·2
	pHBF1BR	75·0		
	pHBBR2	1130		
<i>E</i>	pBR322	32·9	2·23	34·0
	pHBF1BR	73·4		
	pHBBR2	1120		
<i>F</i>	pBR322	22·4	5·09	62·0
	pHBF1BR	114		
	pHBBR2	1390		
<i>H</i>	pBR322	56·8	1·08‡	2·16
	pHBF1BR	61·2		
	pHBBR2	122		
<i>J</i>	pBR322	32·2	1·04‡	2·97
	pHBF1BR	33·6		
	pHBBR2	95·9		
<i>R</i>	pBR322	18·7	1·23‡	15·0
	pHBF1BR	23·0		
	pHBBR2	280		

*All cells used were BW545 λ gt2-derived lysogens with *S. typhimurium met::lacZ* fusion constructs; assays quantified the resulting β -galactosidase activity (Miller, 1972).

†pHBF1BR/pBR322 and pHBBR2/pBR322 represent the ratio of reporter-directed β -galactosidase activity in cells expressing low levels (pHBF1BR) or high levels (pHBBR2) of *in vivo* SAMase activity. Unless noted below, all ratios indicate a statistically significant SAMase-induced *met* gene activity.

‡Not significantly induced (Student's *t* test, $P > 0.05$).

the relative sensitivity of *met* gene promoters to induction under SAM-limiting conditions (Table 4; Greene *et al.*, 1973; Holloway *et al.*, 1970); and in our *metJ::Tn5* (no aporepressor) and *metK::Tn5* (limited SAM synthesis) cells (Table 5; Greene *et al.*, 1973; Su & Greene, 1971).

The current model explains the trends in expression of genes shown to be sensitive to SAM levels and/or the absence of the MetJ aporepressor, but understanding the low but significant SAMase-mediated induction of *metJ* requires more thought. *metJ* is autoregulated, as shown by the repressive effect of excess levels of the aporepressor on *metJ* expression with or without added SAM (Shoeman *et al.*, 1985; Urbanowski & Stauffer, 1986). SAM enhances this autoregulatory activity, so the removal of SAM by SAMase may simply decrease the effectiveness of this regulation, requiring higher concentrations of MetJ to regulate its own gene. This concept is further supported by both the higher level of *metJ* expression in MetJ-deficient cells and the lack of further *metJ* induction in cells lacking the aporepressor (Table 5).

It is more difficult to explain the enhanced induction of all *met* genes in response to SAMase activity in *metJ::Tn5* and *metK::Tn5* backgrounds through the activity of the MetJ–SAM repressor alone. MetJ⁻ cells lack the MetJ aporepressor and, consequently, should fully and constitutively express genes regulated solely by the MetJ–SAM complex. However, cells bearing these Tn5-inactivated *metJ* and *metK* alleles – as well as other *metJ* and *metK* alleles that allow constitutive expression of *met* genes (data not shown) – consistently show enhanced expression of all tested *met* genes in the presence of *in vivo* SAMase activity with the single exception of *metJ* in MetJ-deficient cells noted above. Some or all of the enhanced *met* expression in cells with this particular *metK::Tn5* insertion may be due to residual SAM synthesis that occurs in these cells (e.g. DNA from these cells is fully Dam methylated and SAM is detectable at <5% of wild-type levels; personal observations), and it would be interesting to test the expression of these genes under SAM-limiting conditions known to be lethal (Newman *et al.*, 1998). At the same time the hyperinduction of *met* genes

Table 5. Effect of *in vivo* SAMase activity on *met* gene expression in MetJ[−] and MetK[−] *E. coli*

All cultures were inoculated from independent colonies of freshly transformed cells and incubated overnight in YT medium containing 15 µg tetracycline ml^{−1} at 32 °C with shaking. Numbers represent means of values from at least five cultures for each condition.

<i>met</i> gene*	Plasmid used	Wild-type cells		<i>metJ</i> ::Tn5 cells		<i>metK</i> ::Tn5 cells	
		<i>met</i> activity	<i>met</i> induction†	<i>met</i> activity	<i>met</i> induction†	<i>met</i> activity	<i>met</i> induction†
<i>B</i>	pBR322	41.6	27.2	694	2.46	119	16.8
	pHBBR2	1130		1710		2000	
<i>E</i>	pBR322	32.9	34.0	343	3.12	203	13.4
	pHBBR2	1120		1070		2760	
<i>F</i>	pBR322	22.4	62.0	358	3.10	266	14.8
	pHBBR2	1390		1110		3940	
<i>H</i>	pBR322	56.8	2.16	31.9	1.81	86.1	3.88
	pHBBR2	123		57.8		334	
<i>J</i>	pBR322	32.3	2.97	259	1.36‡	102	1.68
	pHBBR2	95.9		353		172	
<i>R</i>	pBR322	18.7	15.0	250	4.16	71.9	16.7
	pHBBR2	280		1040		1200	

*All cells used were BW545 λ gt2-derived lysogens with *S. typhimurium met::lacZ* fusion constructs; assays quantified the resulting β -galactosidase activity (Miller, 1972).

†‘*met* induction’ represents the ratio of reporter-directed β -galactosidase activity in cells with *in vivo* SAMase activity (transformed with pHBBR2) to negative controls (containing pBR322). Unless noted below, all pHBBR2/pBR322 ratios represent a significant induction of *met* gene activity according to Student’s *t* test at $P < 0.05$.

‡Not significantly induced (Student’s *t* test, $P > 0.05$).

by SAMase suggests a need to dissect the impact of other factors known to regulate *met* gene expression under these conditions and to search for other sources of regulation.

The impact that SAMase-enhanced levels of MetR and/or homocysteine might have on *met* gene expression warrants the most careful consideration. MetR by itself activates *metH* expression (Urbanowski & Stauffer, 1989a; Urbanowski *et al.*, 1987), and a MetR–homocysteine complex in the absence of the MetJ–SAM repressor is needed for efficient expression of *metE* (Plamann *et al.*, 1988; Urbanowski *et al.*, 1987). In addition, MetR[−] cells are impaired to some degree in expression of *metA* and *metF*, each of which is downregulated when homocysteine levels increase, a result presumably of increased levels of the MetR–homocysteine complex and a concomitant decrease of the free MetR activator protein in the cell (Cowen *et al.*, 1993; Mares *et al.*, 1992). It is also possible that MetR, free or bound to homocysteine, is needed in some way for efficient expression of other *met* genes. Consequently, assuming that levels of MetR are normally sufficient only to induce submaximal expression of its target genes, the induction of *metR* directed by SAMase activity could boost expression of MetR-activated genes.

The above logic is contradicted by the observation that *metR* expression is also induced in *metJ*::Tn5 and *metK*::Tn5

cells lacking SAMase. These cells should therefore also exhibit enhanced *met* gene expression comparable to cells with high levels of SAMase if increased levels of MetR synthesis are responsible for the extra level of expression of these genes. SAMase also presumably enhances synthesis of homocysteine through induction of *metA*, *B* and *C*, and this could also play a role in enhancing *met* gene expression. Depending on the fate of any extra homocysteine produced as a consequence of SAMase expression, this should lead to a higher level of the MetR–homocysteine activator complex (and less free MetR). Increased levels both of MetR and homocysteine would presumably also occur in both MetJ- and MetK-deficient cells, both of which are induced for *metA*, *B* and *C* with or without *in vivo* SAMase activity. These and other conflicting and unresolved possibilities surrounding SAMase-mediated induced *met* gene induction can only be resolved by further investigation of the role of MetR activity and/or homocysteine levels, or by searching for other unidentified regulatory elements that influence *met* gene activity such as revealed by the role of SAM in the S-box system of *Bacillus subtilis* (Murphy McDaniel *et al.*, 2003).

Explanations for this effect might also be found outside of the *met* regulon. One possible explanation for the increased expression of *met* genes in *metJ*- and *metK*-deficient cells would include any positive effect on gene transcription

resulting from SAMase-mediated hypomethylation of chromosomal DNA. Methylation of promoter sequences, particularly in studies with -GATC- sequence methylation mediated by the Dam methylase, results in reduced transcription for some genes in *E. coli* (e.g. Stauffer & Stauffer, 1988; reviewed by Palmer & Marinus, 1995; Plumbridge, 1987). Reduction of SAM pools to levels below those needed for Dam- or Dcm-mediated DNA methylation has been achieved with the *in vivo* expression of recombinant SAMase (Hughes *et al.*, 1987; Macintyre *et al.*, 2001; Posnick & Samson, 1999). Should *met* gene promoters or those of genes whose activity impacts *met* gene expression be hypomethylated, or should any other consequence of hypomethylation affect DNA packing or structure, an increase in the transcription of their corresponding genes could result. Answers to these possibilities await studies with Dam- or Dcm-deficient strains.

ACKNOWLEDGEMENTS

This work was supported by VanSant and Faculty Development grants from Ursinus College and by Faculty Development grants from Hanover College. We would like especially to acknowledge the help of former Ursinus College student Kyle Mansfield and former Hanover College students Katie Receuver and Sarah Palecek. *E. coli* K-12 strains BW545, GW2517, GW2521, GW2522, GW2529 and GW2533 were the kind gifts of Graham Walker, MIT, and the λ gt2 *met* gene reporter phage constructs were gratefully received from George Stauffer, University of Iowa.

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