Regulation of the *metR* Gene of *Salmonella typhimurium*

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Regulation of the *Salmonella typhimurium metR* gene was studied by measuring β-galactosidase levels in *Escherichia coli* strains lysogenic for a lambda bacteriophage carrying a *metR-lacZ* fusion. The results indicate that the *metR* gene is negatively regulated by its own gene product and that this autoregulation involves homocysteine as a corepressor. In addition, the results indicate that the *metR* gene is negatively regulated by the *metJ* gene product over a 70- to 80-fold range.

The methionine biosynthetic genes are scattered around the chromosomes of *Escherichia coli* and *Salmonella typhimurium* and form a regulon (9; for a review, see reference 7). Expression of the regulon is controlled by noncoordinate repression of transcription by the *metJ* gene product and S-adenosylmethionine. Recently, it has been shown that the *metR* gene product, a positive-acting protein, is required for expression of the *metE* and *metH* genes (11). These two genes encode the vitamin B_{12}-independent and vitamin B_{12}-dependent homocysteine transmethylases, respectively (7). Both of these enzymes catalyze the last step in methionine biosynthesis.

To understand the role of the *metR* gene product in the regulation of methionine biosynthesis, we have examined the regulation of the *metR* gene itself. Since no assay for the *metR* gene product is available, a *metR-lacZ* gene fusion was constructed and used to study *metR* gene expression. In this system, the synthesis of a functional chimeric β-galactosidase enzyme is directed by the *metR* gene control region.

Construction of λ lysogens carrying a *metR-lacZ* gene fusion. The *metR* gene from *S. typhimurium* has been cloned (11), and its nucleotide sequence has been determined (5). To construct a *metR-lacZ* gene fusion, plasmid pGS191, which carries the *S. typhimurium metR* gene, was digested with restriction enzymes *MluI* and *PstI* (Fig. 1). A 581-base-pair DNA fragment carrying the *metR* control region was isolated, the *MluI* site was filled in with the large fragment of DNA polymerase I (3), and the DNA fragment was partially digested with the restriction enzyme *Sau3AI*. A 458-base-pair DNA fragment carrying the *metR* promoter region and the first 39 codons of the *metR* gene was isolated and ligated into the *lacZYA* fusion vector pMC1403 (1) at the *SmaI* and *BamHI* restriction sites. In the resulting plasmid, designated pRlac, the site of fusion of codon 39 of the *metR* gene to codon 8 of the *lacZ* gene was verified by DNA sequencing. The *metR-lacZ* gene fusion and the *lacY* and *lacA* genes carried on plasmid pRlac were cloned into the single *EcoRI* site in bacteriophage λgt2 by the method described previously (10). The resultant phage, designated λRlac, was plaque purified and was used to lysogenize the *E. coli* strains shown in Table 1. All lysogens were tested to verify that they were single lysogens (8).

The *metR* gene is negatively regulated by the *metJ* repressor. The *metR* gene lies near the *metE* gene in both *S. typhimurium* and *E. coli* (11). In the former, it has been shown that

the two genes are divergently transcribed and that the two promoter elements overlap substantially (Fig. 1 [5]). Since expression of the *metE* gene is subject both to negative control via the *metJ* repressor and to positive control via the *metR* gene product, it seems likely that expression of the overlapping *metR* promoter would also be influenced by these two regulatory gene products. To test whether the *metR* gene was subject to *metJ*-mediated repression, λRlac lysogens were grown in glucose minimal medium plus phenylalanine and vitamin B1 (GMM) supplemented with either L-methionine (repressing condition) or D-methionine (nonrepressing condition), and β-galactosidase levels were assayed (4) as an indication of *metR* gene expression. In the Met$^+$ parent lysogen 162λRlac, addition of L-methionine to the growth medium caused a two- to threefold repression of β-galactosidase activity (Table 2). In contrast, the *metJ* lysogen 597λRlac showed elevated β-galactosidase activity that was not repressed by the addition of L-methionine to the growth medium. These results indicate that the *metR* gene is subject to *metJ*-mediated repression over about a 70- to 80-fold range.

The *metR* gene is negatively autoregulated. Many positive-acting regulatory genes in *E. coli* employ autoregulation as a mechanism to maintain relatively constant levels of gene product in the cell (6). Thus, the *metR-lacZ* fusion phage was used to determine whether the *metR* gene product regulates its own synthesis. In the Met$^-$ *metR* lysogen 244λRlac, β-galactosidase activity was more than fourfold higher than in the Met$^-$ *metE* control lysogen 243λRlac when the cells were grown in GMM plus D-methionine (Table 2), suggesting that the *metR* gene is involved in negatively regulating its own expression. Although the control lysogen 243λRlac and the *metR* lysogen 244λRlac are both functionally *metE* mutants (11), their *metE* alleles are different. To rule out the possibility that the differences seen are due to heterogenous *metE* alleles, we tested lysogens 705λRlac (*metE163*::Tn*10*) and 750λRlac (*metE163*::Tn*10 metR*) under the same conditions and found a similar four- to fivefold negative autoregulation (Table 2). Interestingly, the addition of L-methionine to the growth medium caused repression of β-galactosidase synthesis in both the 244λRlac and 750λRlac lysogens, indicating that negative control by the *metJ* repressor can override derepression caused by the *metR* mutation.

Since the *metJ* repressor negatively regulates *metR* gene expression, the apparent negative regulation of *metR* by its own gene product could actually be an indirect result of positive regulation of the *metJ* gene by the *metR* gene product. This hypothesis was tested in two ways. First, we
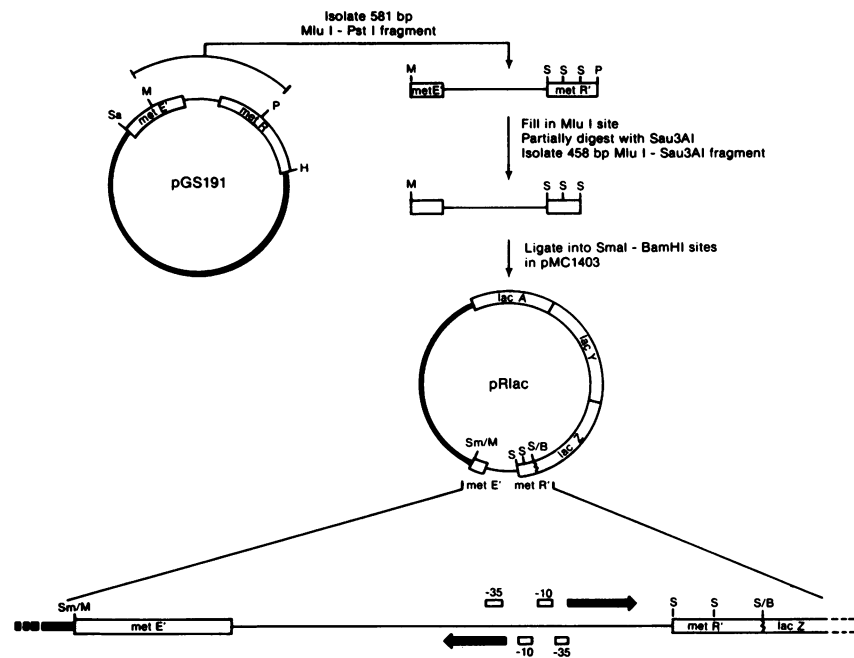
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FIG. 1. Construction of the *metR-lacZ* gene fusion. The fusion consists of a 458-base-pair *Mlu*I-*Sau*3AI DNA fragment containing the *metE-metR* control region of *S. typhimurium* from plasmid pGS191 (11) fused at codon 39 of the *metR* gene to codon 8 of the *E. coli lacZ* gene. Synthesis of the chimeric β-galactosidase product was dependent on transcription and translation initiation from the *metR* control region. The −10 and −35 regions of the rightward-transcribed *metR* promoter and leftward-transcribed *metE* promoter overlap substantially. Heavy lines indicate plasmid pBR322 vector DNA. Open boxes indicate structural gene segments; the primed gene symbol denotes a truncated gene. Heavy arrows indicate the directions of transcription of the *metE* and *metR* genes. Abbreviations: B, *Bam*HI; H, *Hin*dIII; M, *Mlu*I; P, *Pst*I; S, *Sau*3AI; Sa, *Sal*I; Sm, *Sma*I.

compared the β-galactosidase levels in the *metJ* lysogen 597λRlac and the *metR* lysogen 244λRlac with those in the *metJ metR* lysogen 748λRlac (Table 2). In lysogen 748λRlac, β-galactosidase levels were two- to threefold higher than in lysogen 597λRlac, indicating that a *metR* mutation could cause derepression of the *metR* gene beyond that caused by inactivation of the *metJ* system. Conversely, in lysogen 244λRlac, where no *metR* gene product is produced, there was enough *metJ* repressor present to repress β-galactosidase synthesis when methionine was added to the growth medium (Table 2). Second, we determined the direct effect of the *metR* mutation on *metJ* gene expression by comparing

β-galactosidase levels in lysogens carrying the *metJ-lacZ* fusion phage λJlac (10). The *metR* lysogen 244λJlac showed no significant difference from the control *metE* lysogen 243λJlac when the cells were grown in GMM supplemented with either D-methionine or L-methionine (Table 2). Together, these results indicate that the *metR* protein is not involved in the regulation of *metJ* gene expression and that the *metR* gene is negatively regulated directly by its own gene product and by the *metJ* repressor.

Involvement of homocysteine in *metR* autoregulation. The *metR*-mediated activation of the divergently transcribed *metE* gene involves the methionine intermediate homocys-

TABLE 1. *E. coli* strain descriptions and origins

Strain	Relevant markers[a]	Source
GS162	Δ*lacU169*	G. Zurawski
GS243	Δ*metE*::Mu Δ*lacU169*	This laboratory
GS244	Δ*metR*::Mu Δ*lacU169*	This laboratory
GS597	*metJ97* Δ*lacU169*	This laboratory
GS705	*metE163*::Tn*10* Δ*lacU169*	This laboratory
GS719	*metB1 metJ97* Δ*lacU169*	This laboratory
GS720	*metB1 metC162*::Tn*10 metJ97* Δ*lacU169*	This laboratory
GS723	*metB1 metC162*::Tn*10 metJ97* Δ*metF*::Mu Δ*lacU169*	This laboratory
GS747	*metB1 metE163*::Tn*10 metJ97* Δ*lacU169*	This laboratory
GS748	*metJ97* Δ*metR*::Mu Δ*lacU169*	This laboratory
GS750	*metE163*::Tn*10* Δ*metR*::Mu	This laboratory
GS753	*metB1 metC162*::Tn*10 metJ97* Δ*metF*::Mu *metR* Δ*lacU169*	This laboratory

[a] In addition to the relevant markers, all strains carry the *pheA905*, *araD129*, *rpsL*, and *thi* mutations.

TABLE 2. Effects of the *metJ* and *metR* gene products on expression of the *S. typhimurium metR-lacZ* gene fusion

Lysogen	β-Galactosidase activity[a]		
	D-Methionine	L-Methionine	D-Methionine + B$_{12}$
162λRlac (Met$^+$)	50	20	80
597λRlac (*metJ*)	1,680	1,410	2,630
243λRlac (*metE*)	100	20	90
244λRlac (*metR*)	440	30	320
748λRlac (*metJ metR*)	3,850	4,100	3,820
705λRlac (*metE*)	70	20	80
750λRlac (*metE metR*)	340	30	260
243λJlac (*metE*)	170	90	ND[b]
244λJlac (*metR*)	160	90	ND

[a] Units of specific activity are nanomoles of *O*-nitrophenol produced per minute per milligram of protein at 28°C. The growth medium was GMM, supplemented as indicated with either D-methionine (150 μg/ml), L-methionine (50 μg/ml), or D-methionine plus vitamin B$_{12}$ (1 μg/ml).
[b] ND, Not done.

teine as a coactivator; O-succinylhomoserine, cystathionine, 5-methyltetrahydrofolate (5-mTHF), and methionine have no effect (M. L. Urbanowski and G. V. Stauffer, manuscript in preparation). We therefore tested the effect of homocysteine on the expression of the metR-lacZ gene fusion. The metJ metB metC lysogen 720λRlac and the metJ metB metC metF lysogen 723λRlac are defective in synthesis of homocysteine, although both strains, when supplemented with limiting amounts of methionine (D-methionine), produce small amounts of homocysteine from endogenous S-adenosylmethionine via a regenerative pathway (2). Lysogen 720λRlac had high β-galactosidase activity when grown in GMM supplemented only with D-methionine (Table 3). However, when homocysteine was added to the growth medium, β-galactosidase activity was reduced nearly 10-fold. A similar reduction was seen in the metJ lysogen 597λRlac, although the effect was less dramatic because of endogenous homocysteine production. This reduction was not seen in the metJ metR lysogen 748λRlac, suggesting that homocysteine acts as a corepressor in metR autoregulation. In contrast, the metF mutation (which blocks 5-mTHF synthesis) in lysogen 723λRlac prevented the high β-galactosidase expression seen in 720λRlac, which suggests two possibilities: (i) either the metF gene product or 5-mTHF is directly required for metR gene expression, or (ii) since homocysteine facilitates repression of metR, 5-mTHF increases metR expression indirectly by allowing utilization of homocysteine via the homocysteine transmethylase reaction, thus preventing accumulation of homocysteine formed through the regenerative pathway discussed above. To distinguish between these two possibilities, we tested metR-lacZ expression in the metJ metB lysogen 719λRlac and in the metJ metB metE lysogen 747λRlac. In 747λRlac the metE mutation prevents utilization of homocysteine and 5-mTHF and thus leads to an accumulation of both intermediates. If 5-mTHF is required directly for expression of the metR-lacZ fusion, then β-galactosidase levels should be intermediate to high in 747λRlac, similar to the levels in 720λRlac. Conversely, if expression responds only to homocysteine, then β-galactosidase levels should be low, similar to the levels in 723λRlac. As shown in Table 3, lysogen 747λRlac had low β-galactosidase levels, suggesting that the low levels seen in 723λRlac were a result of homocysteine repression due to an accumulation of this

intermediate and that 5-mTHF acted indirectly by affecting the homocysteine pools.

The simplest model for homocysteine repression of metR is one in which this intermediate acts as a corepressor with the metR protein rather than acting by a metR-independent mechanism. If this model is correct, then it should be possible to isolate metR mutants of 723λRlac on lactose minimal medium showing high derepressed β-galactosidase levels. To test this hypothesis, portions (0.05 ml at 2×10^9 cells per ml) of four independent overnight cultures of lysogen 723λRlac were plated onto lactose minimal plates supplemented with phenylalanine, vitamin B_1, D-methionine, and 1 mM phenylethyl-β-D-thiogalactoside (a lactose analog that reduces background growth of lysogen 723λRlac). Lysogen 723λRlac grew very slowly on this medium. From each selection plate, one Lac+ colony which arose after 48 h was purified and the β-galactosidase levels were measured. All four independently isolated mutants showed similar derepressed β-galactosidase activity. The enzyme levels of one representative mutant, 753λRlac, are shown in Table 3. The new mutation in 753λRlac, and in each of the other three mutants, was shown to lie in the metR gene by the following three criteria. (i) All four mutants, when transformed with a single-copy-number plasmid carrying only the metR gene (plasmid pGSmetR), showed low β-galactosidase levels, indicating complementation of the mutations in trans by the metR plasmid [only 753λRlac-(pGSmetR) is shown in Table 3]. (ii) All four mutations were linked to the ilv locus by phage P1 transduction (data not shown), consistent with the map position of metR (11). (iii) New ilv+ metR transductants isolated in the above P1 experiments, when lysogenized with a λElac phage carrying a metE-lacZ fusion, show greatly reduced levels of metE-lacZ expression (data not shown), typical of a metR mutant (11).

Involvement of vitamin B_{12} in metR regulation. We tested whether vitamin B_{12} influenced expression of the metR gene. In both the wild-type lysogen 162λRlac and the metJ lysogen 597λRlac, a small increase in β-galactosidase levels was seen when vitamin B_{12} was added to the growth medium (Table 2). It is not clear whether metR gene expression is directly enhanced by the metH holoenzyme or whether this enhanced expression is an indirect result of a turning off of the overlapping metE promoter by the metH holoenzyme.

TABLE 3. Effects of homocysteine and the metF gene product on expression of the metR-lacZ gene fusion

Lysogen	Relevant genotype	β-Galactosidase activity[a]	
		D-Methionine	D-Methionine + homocysteine
720λRlac	metJ metB metC	4,770	500
723λRlac	metJ metB metC metF	290	210
597λRlac	metJ	1,750	250
748λRlac	metJ metR	4,350	3,850
719λRlac	metJ metB	3,070	430
747λRlac	metJ metB metE	330	260
753λRlac	metJ metB metC metF metR	4,500	3,890
753λRlac (pGSmetR)	metJ metB metC metF metR/metR+	250	210

[a] Units of specific activity are nanomoles of O-nitrophenol produced per minute per milligram of protein at 28°C. The growth medium was GMM, supplemented where indicated with D-methionine (150 μg/ml) or D-methionine plus DL-homocysteine (100 μg/ml).

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