MetJ-mediated regulation of the *Salmonella typhimurium* metE and metR genes occurs through a common operator region

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(Received 16 November 1992; revision received 5 January 1993; accepted 6 January 1993)

Abstract: In *Salmonella typhimurium* the metE and metR promoters overlap and are divergently transcribed. Three tandem repeats of an 8 bp sequence defined previously as the metE operator site for MetJ-mediated repression also overlap the −35 region of the metR promoter. Starting with a metE-lacZ·metR-galK double gene fusion, site-directed mutagenesis was used to change nucleotides in each of the repeat units from the consensus sequence. Each mutation, along with the wild-type metE-lacZ·metR-galK gene fusion, was cloned into phage Agt2. Regulation of the metE and metR genes was examined by measuring β-galactosidase and galactokinase levels in *Escherichia coli* strains lysogenized with phage carrying the wild-type and mutant fusions. Mutations in each of the 8 bp repeat units disrupt MetJ-mediated repression for both the metE-lacZ and metR-galK gene fusions, suggesting that the metE and metR genes share a common operator site for the MetJ repressor.

Key words: *Salmonella typhimurium*; MetJ-mediated regulation; metE-metR operator region; Site-directed mutagenesis

Introduction

In *E. coli* and *S. typhimurium*, expression of the *met* regulon, encoding the methionine biosynthetic enzymes, involves both negative and positive control of transcription [1]. Positive control is mediated by the metR gene product, a DNA-binding protein [2,3] that activates both metE and metH expression [2,4,5]. Homocysteine, a pathway intermediate, modulates this activation by stimulating metE expression and inhibiting metH expression [6,7]. Negative control is mediated by the metJ gene product, with S-adenosylmethionine (SAM) as the co-repressor [1,8]. The MetJ repressor is thought to function by binding as a dimer to an eight basepair (bp) DNA recognition site, with a consensus sequence of 5′-AGACGTCT-3′ [9,10]. Degenerate forms of the eight bp consensus sequence occur as tandemly repeated units of from two to five repeats in the regulatory regions of the different met genes [9].

The promoters for the metE and metR genes overlap and are transcribed in opposite directions [2,11]. In the metE-metR regulatory region of *S. typhimurium* there are three tandem repeats of
the eight bp MetJ binding sequence which overlap the -35 region of the metR promoter and are just downstream from the -10 region of the metE promoter (Fig. 1). Operator-constitutive mutations for the metE gene in S. typhimurium were previously found to be located in two of the three tandem eight bp repeat units [12]. We demonstrate here that mutations in all three tandem repeats interfere with repression of both metE and metR gene expression, suggesting a common operator site for MetJ-mediated repression.

Materials and Methods

Bacterial strains

Strains used are listed in Table 1. The ΔgalK-Σtet-50 allele is a deletion of the galK gene and its substitution (Σ) by the tet gene from pBR322 [3]. The ΔgalK-Σtet-50 allele was introduced into appropriate strains by transduction using P1 clr-100 phage [13]. All strains are isogenic except JM107 and BW313.

Media

Luria broth, Luria agar and glucose minimal medium (GM) have been described [14]. GM was supplemented with phenylalanine and vitamin B1 since most strains used in this study carry the pheA905 and thi markers. Supplements and antibiotics were added at the following concentrations: amino acids, 50 μg/ml; vitamin B1, 1 μg/ml; ampicillin, 100 μg/ml; tetracycline, 3 μg/ml.

Oligonucleotide-directed mutagenesis

A 1.5 kb EcoRI-BamHI DNA fragment containing the metE-metR control region and the beginning of both structural genes was isolated from plasmid pElac-Rgal [3] and ligated into the EcoRI and BamHI sites of phage M13mp18 (designated M13mp18Elac-Rgal). The oligonucleotide-directed mutagenesis method of Kunkel [15] was then used to construct mutations in the recombinant phage. DNA sequencing [16] was used to screen for the desired mutations.

Lysogen construction

Replicative form DNA from each M13mp18-Elac-Rgal phage carrying a base change was prepared [17] and digested with EcoRI and BamHI. The 1.5 kb fragment was isolated from a low melting temperature agarose gel and ligated into the EcoRI and BamHI sites of pMC1403 [18]. This resulted in an in-frame fusion of the 22nd codon of metE and the 8th codon of the lacZ gene in plasmid pMC1403. The pMC1403-mutant plasmids and the wild-type pElac-Rgal [3] were digested with StuI, ligated with EcoRI linkers and recut with EcoRI. The EcoRI DNA fragment of 7 kb generated from each digestion carries the metR-galK and metE-lacZ fusions. This fragment from each digestion was digested and ligated into the unique EcoRI site in phage λgt2. The ligated DNA was then packaged into λ phage particles [19]. Appropriate strains were lysogenized with gElac-Rgal phage carrying wild-type or operator-constitutive mutations, and the lysogens were tested for a single copy of λ phage as described [19]. All λ lysogens have the temperature sensitive mutation c1857 and were grown at 30°C.

Enzyme assays

β-galactosidase was assayed as described by Miller [13]. Galactokinase was assayed as described by McKenney et al. [20]. All results are averages of two or more experiments in which each sample was assayed in triplicate. Ranges of activities between experiments are within 30% of the average values given.
Results and Discussion

Effect of operator mutations on metE-lacZ and metR-galK expression

The MetJ binding region for the metE gene consists of three tandem repeats of the met box sequence (Fig. 1). Bases in each repeat were changed away from consensus in the operator region on a metE-lacZ·metR-galK double gene fusion and the mutated gene fusions were cloned into phage λgt2 (see Materials and Methods). The mutant phages were designated θElac·Rgal-24G, θElac·Rgal-37C, θElac·Rgal-42ΔG, and θElac·Rgal-45A. The λgt2 phage carrying the wild-type Elac·Rgal fusion and the mutated derivatives were used to lysogenize strain GS966 (metE). The resulting lysogens were designated 966AElac·Rgal, 966AElac·Rgal-24G, 966AElac·Rgal-37C, 966AElac·Rgal-42ΔG, and 966AElac·Rgal-45A. Lysogens were grown in GM supplemented with either L-methionine (repressing condition) or D-methionine (derepressing condition), and β-galactosidase levels were measured. D-methionine is converted to L-methionine by the cell and serves as a methionine-limiting condition [21]. All mutant lysogens showed elevated β-galactosidase levels compared to the wild-type lysogen when grown in the presence of L-methionine (Table 2). β-galactosidase levels were further increased in the wild-type and the mutant lysogens when grown in the presence of D-methionine. In the wild-type lysogen, the repressed and derepressed β-galactosidase levels varied over an 8- to 9-fold range, whereas in the mutant lysogens the levels varied over a 2.1- to 2.7-fold range. These results are similar to those reported previously for metE-lacZ operator-constitutive mutants [12].

To test the effects of the mutations on metR gene expression, galactokinase levels were also measured in the same lysogens grown in GM supplemented with either L-methionine or D-methionine. In the lysogen 966AElac·Rgal, galactokinase levels were repressed 3-fold by the addition of L-methionine to the growth medium (Table 2). All of the mutant lysogens showed elevated galactokinase levels in the D-methionine cultures, and the levels were poorly repressed by L-methionine addition. Thus, changing bases in any of the three met box sequences in the metE-metR control region interferes with normal methionine-mediated repression of the metR-galK fusion. These results suggest that the previously defined operator site for the metE gene [12] also regulates metR gene expression.

Effect of operator mutations in a metJ mutant

To show that the altered expression of both the metE-lacZ and metR-galK fusions in the mutant lysogens is due to the loss of MetJ-mediated

![Fig. 1. Location of changes in the MetJ binding site of the metE-metR regulatory region. Nucleotide numbering is according to Plamann and Stauffer [11]. The start sites of transcription and the −10 and −35 regions for metR and metE were determined previously [11], and are indicated on the top and bottom strands, respectively. The region of DNA protected from DNase I digestion by bound MetR protein [3] is indicated by the bracket. Three tandem repeats of the consensus MetJ binding sequence are indicated above the DNA sequence. Dots indicate bases that are identical to the consensus 8 bp repeat unit 5′-AGACGTCT-3′. Base changes made by oligonucleotide-directed mutagenesis are shown below the sequence. All changes are different from the consensus for MetJ binding. Numbering of the mutations is relative to the transcription initiation site (+1) for metR.](https://academic.oup.com/femsle/article-abstract/108/2/145/588731)
repression, we lysogenized the metE metI mutant strain GS969 with phages carrying the wild-type and mutant operator sequences. The resulting lysogens, 969AElac- Rgal, 969AElac- Rgal-24G, 969AElac· Rgal-37C, 969AElac· Rgal-42ΔG, and 969AElac· Rgal-45A, were grown in GM supplemented with either L-methionine or D-methionine, and both β-galactosidase and galactokinase levels were measured. In the control metI lysogen 969AElac· Rgal, both β-galactosidase and galactokinase activities were elevated and poorly repressed by methionine (Table 3) compared to the metI+ lysogen 966AElac· Rgal (Table 2). Similarly, in the metI background the galactokinase levels of all of the mutant fusions were not significantly different than the level for the wild-type fusion. These results suggest that the altered regulation of the operator mutants seen in the metI+ background is metI-dependent.

**Effects of operator mutations in a metI metR mutant**

Although none of the base changes in the operator region alter the -10 and -35 regions of either the metE or the metR promoter, they are located close enough to the promoters to possibly affect promoter strength. To determine whether any of the base changes alter the metE or metR promoters, strain GS968 (metJ metR) was lysogenized with wild-type and mutant gElac· Rgal phages. The lysogens were designated 968AElac· Rgal, 968AElac· Rgal-24G, 968AElac· Rgal-37C, 968AElac· Rgal-42ΔG, and 968AElac· Rgal-45C. Since the metR gene is negatively autoregulated

### Table 2

Effects of metE operator mutations on metE-lacZ and metR-galK expression in a metE mutant

<table>
<thead>
<tr>
<th>Lysogen a</th>
<th>β-galactosidase activity b</th>
<th>Galactokinase activity c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-met</td>
<td>D-met</td>
</tr>
<tr>
<td>966AElac· Rgal</td>
<td>378</td>
<td>3285</td>
</tr>
<tr>
<td>966AElac· Rgal-24G</td>
<td>1518</td>
<td>4184</td>
</tr>
<tr>
<td>966AElac· Rgal-37C</td>
<td>2704</td>
<td>5386</td>
</tr>
<tr>
<td>966AElac· Rgal-42ΔG</td>
<td>1413</td>
<td>3898</td>
</tr>
<tr>
<td>966AElac· Rgal-45A</td>
<td>2478</td>
<td>4264</td>
</tr>
</tbody>
</table>

a All lysogens were grown in GM supplemented with either L-methionine or D-methionine, as indicated. b Units are Miller units of activity [13]. c Units are nanomoles of galactose phosphorylated at 30°C per min per ml of cells at an optical density at 650 nm of 1.

### Table 3

Effect of operator mutations on metE-lacZ and metR-galK expression in a metE metI mutant

<table>
<thead>
<tr>
<th>Lysogen a</th>
<th>β-galactosidase activity b</th>
<th>Galactokinase activity c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-met</td>
<td>D-met</td>
</tr>
<tr>
<td>969AElac· Rgal</td>
<td>8091</td>
<td>7762</td>
</tr>
<tr>
<td>969AElac· Rgal-24G</td>
<td>7509</td>
<td>7602</td>
</tr>
<tr>
<td>969AElac· Rgal-37C</td>
<td>8880</td>
<td>9188</td>
</tr>
<tr>
<td>969AElac· Rgal-42ΔG</td>
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<td>6899</td>
</tr>
<tr>
<td>969AElac· Rgal-45A</td>
<td>8219</td>
<td>7721</td>
</tr>
</tbody>
</table>

a All lysogens were grown in GM supplemented with either L-methionine or D-methionine, as indicated. b Units are Miller units of activity [13]. c Units are nanomoles of galactose phosphorylated at 30°C per min per ml of cells at an optical density at 650 nm of 1.
as well as negatively regulated by the MetJ protein [8], the galactokinase levels in the GS968 lysogens should reflect the intrinsic strength of the metR promoter in the absence of both regulatory proteins. The metE gene is negatively regulated by the MetJ protein and positively regulated by the MetR protein [1,5]. Similarly, β-galactosidase levels in the GS968 lysogens should reflect the strength of the metE promoter in the absence of both proteins. The lysogens were grown in GM supplemented with L-methionine or D-methionine, and β-galactosidase and galactokinase levels were measured. The galactokinase activity for the 968ΔElac · Rgal lysogen and the four mutant lysogens were all similarly elevated and nonrepressible by methionine (Table 4), suggesting that the mutations are not promoter-up mutations or promoter-down mutations for metR gene expression.

The β-galactosidase levels for the parental 968ΔElac · Rgal lysogen and the mutant lysogens are all significantly reduced due to the absence of the MetR activator protein (Table 4). If any of the mutations affect the metE promoter, one might expect a change in the basal level of metE-lacZ expression relative to the parent lysogen, even in the absence of MetR. Although the β-galactosidase levels in two of the mutant lysogens (968ΔElac · Rgal-37C and -45A) are essentially the same as the parental lysogen, β-galactosidase in the other two mutant lysogens (-24G and -42ΔG) are lower. These results suggest that the mutations -24G and -42ΔG have slight negative effects on metE promoter strength.

It should be noted that the galactokinase levels are 6- to 11-fold higher in the metJ metR double mutant than in the metJ mutant (compare Table 4 and Table 3). These results are consistent with previous results [3] suggesting that the metE and metR promoters are reciprocally regulated.

MetJ directly mediates repression of metE and metR

The metE gene is activated by the the MetR protein [5]. Since the metR gene is negatively regulated by MetJ, it is possible that MetJ-mediated repression of the metE gene is due to a decrease in MetR levels rather than by direct repression of metE. This mechanism is unlikely, however, for several reasons. As shown in Table 2, MetJ-mediated repression of the metE gene is much greater in the 966ΔElac · Rgal wild-type lysogen than the mutant lysogens. It should be noted that MetR levels are determined by the single wild-type copy of the chromosomal metR gene, and should be essentially the same in all of the GS966 lysogens. If MetJ-mediated regulation of the metE gene was indirect, occurring solely by controlling the levels of MetR protein, then β-galactosidase levels would be expected to be the same in the 966ΔElac · Rgal wild-type and mutant lysogens. In addition, we have measured metE-lacZ expression in a metR mutant. Although metE-lacZ expression is decreased due to the absence of the MetR protein, MetJ-mediated repression still occurs over a 10-fold range (unpublished results). These results suggest that a major part of the negative regulation of metE expression is by direct MetJ-mediated repression.

Table 4

<table>
<thead>
<tr>
<th>Lysogen a</th>
<th>β-galactosidase activity b</th>
<th>Galactokinase activity c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-met</td>
<td>D-met</td>
</tr>
<tr>
<td>968ΔElac · Rgal</td>
<td>196</td>
<td>175</td>
</tr>
<tr>
<td>968ΔElac · Rgal-24G</td>
<td>141</td>
<td>130</td>
</tr>
<tr>
<td>968ΔElac · Rgal-37C</td>
<td>189</td>
<td>186</td>
</tr>
<tr>
<td>968ΔElac · Rgal-42ΔG</td>
<td>133</td>
<td>115</td>
</tr>
<tr>
<td>968ΔElac · Rgal-45A</td>
<td>202</td>
<td>213</td>
</tr>
</tbody>
</table>

a All lysogens were grown in GM supplemented with either L-methionine or D-methionine, as indicated. b Units are Miller units of activity [13]. c Units are nanomoles of galactose phosphorylated at 30°C per min per ml of cells at an optical density at 650 nm of 1.
Similarly, MetJ probably directly represses metR expression. As mentioned above, the metE and metR promoters are reciprocally regulated, and changes that increase metE expression decrease metR expression, whereas changes that increase metR expression decrease metE expression [3]. If MetJ indirectly repressed metR by controlling the rate of transcription initiation from the competing metE promoter, then mutations that increase metE expression would be expected to decrease metR expression. As shown in Table 2, base changes in the 8 bp met box sequences increase both metE and metR expression, suggesting that MetJ directly represses metR.

**Acknowledgement**

This study was supported by Public Health Service grant GM38912 from the National Institute of General Medical Sciences.

**References**