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# Autoregulation by Tandem Promoters of the *Salmonella typhimurium* LT2 *metJ* Gene

MARK L. URBANOWSKI AND GEORGE V. STAUFFER*
*Department of Microbiology, University of Iowa, Iowa City, Iowa 52242*

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**Regulation of the *Salmonella typhimurium metJ* gene was examined by measuring β-galactosidase activity in *Escherichia coli* strains lysogenic for a phage carrying a *metJ-lacZ* gene fusion. The results indicated that the *metJ* gene is regulated by its own gene product and by methionine supplementation to the growth medium. This autoregulatory mechanism involved two tandem promoters, $p_{J1}$ and $p_{J2}$, separated by approximately 65 base pairs. Deletion analysis permitted the assessment of the activity of promoters $p_{J1}$ and $p_{J2}$ individually. Promoter $p_{J1}$ was negatively regulated by the *metJ* gene product and by methionine. Although $p_{J2}$ regulation remained unclear, evidence is presented which suggests that it is not negatively regulated like $p_{J1}$.**

In both *Salmonella typhimurium* and *Escherichia coli* the *metJ* gene codes for a protein that is involved in the regulation of the methionine pathway (5, 13, 17). This protein interacts with *S*-adenosylmethionine to form an active complex that presumably functions as a classical repressor (13, 17). The *metJ* gene of *S. typhimurium* has been cloned and its nucleotide sequence has been determined (18, 19). To facilitate studies on regulation of the *metJ* gene, we have fused the upstream control sequences for this gene to the *E. coli lacZ* gene. Deletion derivatives of this fused gene have been constructed to test a model proposing that the *metJ* gene is transcribed from two distinct interacting promoters (19).

## MATERIALS AND METHODS

**Bacterial strains, phage, and plasmids.** Descriptions and origins of bacterial strains are given in Table 1. All bacterial strains are derivatives of *E. coli* K-12. Plasmid pGS107 was described previously (18). Plasmid pMC1403 (2) was from M. Casadaban. Phage λgt2 (10) was from R. Davis. Other plasmids and phage were isolated during this investigation.

**Media.** Luria broth and glucose minimal media have been described (16). Supplements were added at the following concentrations: phenylalanine, 50 μg/ml; vitamin $B_1$, 1 μg/ml; D-methionine, 150 μg/ml; L-methionine, 50 μg/ml; ampicillin, 100 μg/ml; 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), 40 μg/ml.

**DNA manipulations.** The general procedures used for restriction enzyme cleavage, ligation, plasmid and phage DNA isolation, isolation of DNA fragments from polyacrylamide gels, and transformation have been described (6). DNA sequence analysis was done by the method of Maxam and Gilbert (7).

**Construction of plasmids pBlac and pJlac.** A 480-base pair (bp) *Rsa*I DNA fragment isolated from plasmid pGS107 that carries the promoters and the coding sequences for the amino-terminal ends of the *S. typhimurium metB* and *metJ* genes was ligated in both orientations into the *Sma*I site of plasmid pMC1403 (Fig. 1). The *Rsa*I cleavage sites occur between codons in the *metB* and *metJ* genes and therefore maintain the reading frame of the *lacZ* gene in plasmid pMC1403. The ligation products were then used to transform strain GS563, the cells were plated on Luria broth-ampicillin plates containing X-gal, and blue colonies were isolated. Plasmid DNA isolated from several blue transformants was analyzed by restriction enzyme digestion to determine the orientation of the 480-bp *Rsa*I fragment. Both *metB-lacZ* and *metJ-lacZ* fusion plasmids were isolated, and the DNA was sequenced to verify the fusion points (not shown). The resultant plasmids, designated pBlac and pJlac, code for two chimeric proteins. The MetB-LacZ protein is a fusion of the first 31 amino-terminal amino acids of the *metB* gene product to the eighth amino acid of β-galactosidase. The MetJ-LacZ protein is a fusion of the first 41 amino-terminal amino acids of the *metJ* gene product to the eighth amino acid of β-galactosidase.

**Construction of plasmid pJlacΔ$P_{J1}$.** The 490-bp *Eco*RI-*Bam*HI DNA fragment from plasmid pJlac carrying the *metB* and *metJ* control regions was isolated and digested completely with restriction enzyme *Aha*II and digested partially with restriction enzyme *Hin*PI. The appropriate fragments were isolated from a polyacrylamide gel and religated, deleting the internal *Aha*II-*Hin*PI fragment and resulting in a fusion of base −83 to +6 (Fig. 2). This fused fragment was then religated into its original plasmid vector pMC1403 and used to transform strain GS563. The deletion endpoints were verified by DNA sequencing (not shown).

**Construction of plasmid pJlacΔ$P_{J2}$.** The method of construction of pJlacΔ$P_{J2}$ was similar to that of pJlacΔ$P_{J1}$ except that an internal *Sau*96I DNA fragment was deleted, resulting in a fusion of base +36 to +72 (Fig. 2).

**Construction of plasmid pBlacΔ$P_{J2}$.** Construction of pBlacΔ$P_{J2}$ deleted the same internal *Sau*96I fragment as was deleted in pJlacΔ$P_{J2}$, but the parent plasmid used was pBlac

TABLE 1. Bacterial strain descriptions and origins

| Strain | Relevant markers | Other markers | Source |
|---|---|---|---|
| GS162 | *ΔlacU169* | *pheA905 araD129 rpsL thi* | G. Zurawski |
| GS243 | *metE ΔlacU169* | *pheA905 araD129 rpsL thi* | This lab |
| GS245 | *glyA ΔlacU169* | *pheA905 araD129 rpsL thi* | This lab |
| GS563 | *recA glyA ΔlacU169* | *pheA905 araD129 rpsL thi* | This lab |
| GS597 | *metJ97 ΔlacU169* | *pheA905 araD129 rpsL thi* | This lab |

* Corresponding author.

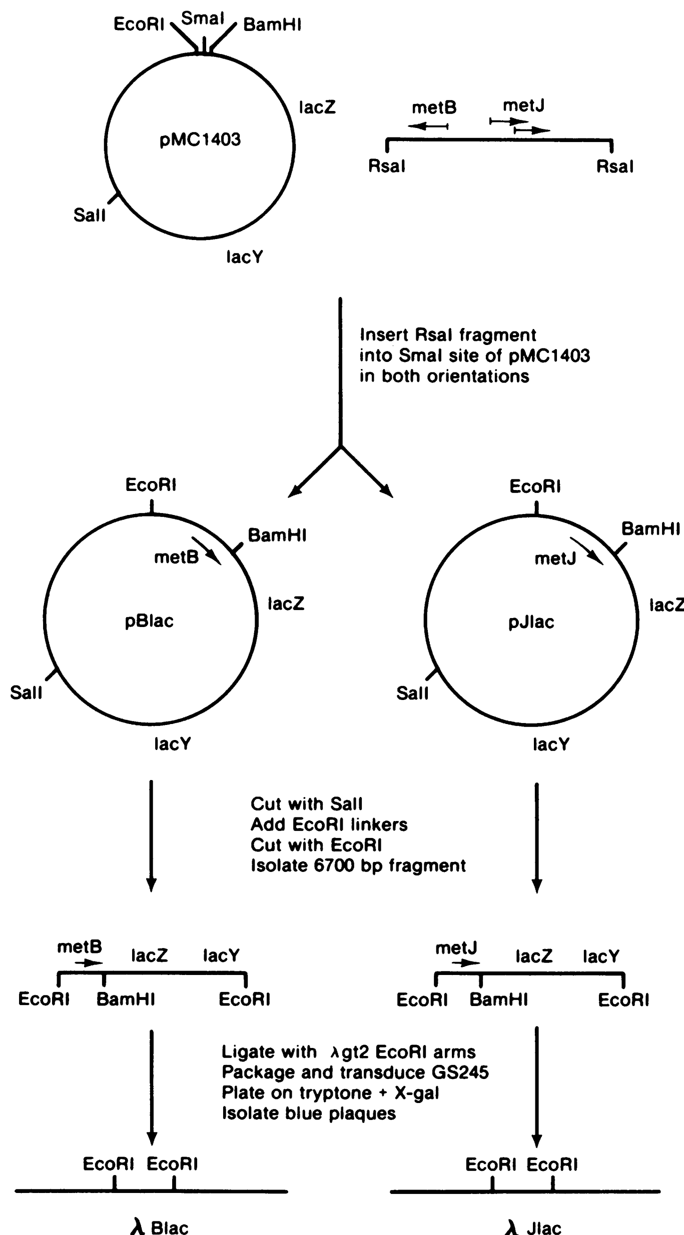


FIG. 1. Construction of the *metB-lacZ* and *metJ-lacZ* gene fusions. A 480-bp *RsaI* fragment from plasmid pGS107 carrying the promoter regions for the *metB* and *metJ* genes was cloned in both orientations into the *SmaI* site of the plasmid vector pMC1403. The arrows indicate the start sites and direction of transcription of the *metB* and *metJ* genes (19). The 6,700-bp *Eco*RI-*SalI* fragment that carries the *metB-lacZ* or *metJ-lacZ* fusion was then inserted in single copy into the phage vector λgt2, giving rise to phage λBlac and λJlac, respectively. Figure is not drawn to scale.

and thus yielded a *metB-lacZ* fusion containing a deletion of the *metJ* promoter $p_{J2}$.

**Construction of the *lacZ* fusion phage.** Plasmids constructed above were cleaved at the unique *SalI* site in the pMC1403 vector, and the protruding ends were filled in with deoxynucleotide triphosphates and the Klenow fragment of *E. coli* DNA polymerase I. *Eco*RI linkers were ligated to the blunted ends, and the plasmids were digested with *Eco*RI. The resultant DNA fragments were run on a low-melting-temperature agarose gel, and the 6,700-bp fragment that carries the *lacZ* fusion and the *lacY* gene was isolated. This 6,700-bp fragment was then ligated with *Eco*RI-cleaved λgt2 phage DNA, and the mixture was packaged by using a commercial lambda-packaging system according to the manufacturer's instructions. Packaging mixtures were used to infect strain GS245, and the cells were plated in tryptone soft agar plus X-gal. Blue plaques were picked and the phage were plaque purified. The phage are designated according to the plasmid DNA used in their construction; e.g., λJlac was constructed with plasmid pJlac DNA; λBlac, with plasmid pBlac DNA, etc. Lysogens were isolated by putting a drop of phage suspension on a soft-agar overlay of an appropriate host and picking phage-resistant colonies from the center of the zone of lysis. Lysogens were then single colony purified on Luria broth–X-gal plates.

**S1 nuclease mapping.** The S1 nuclease mapping procedure

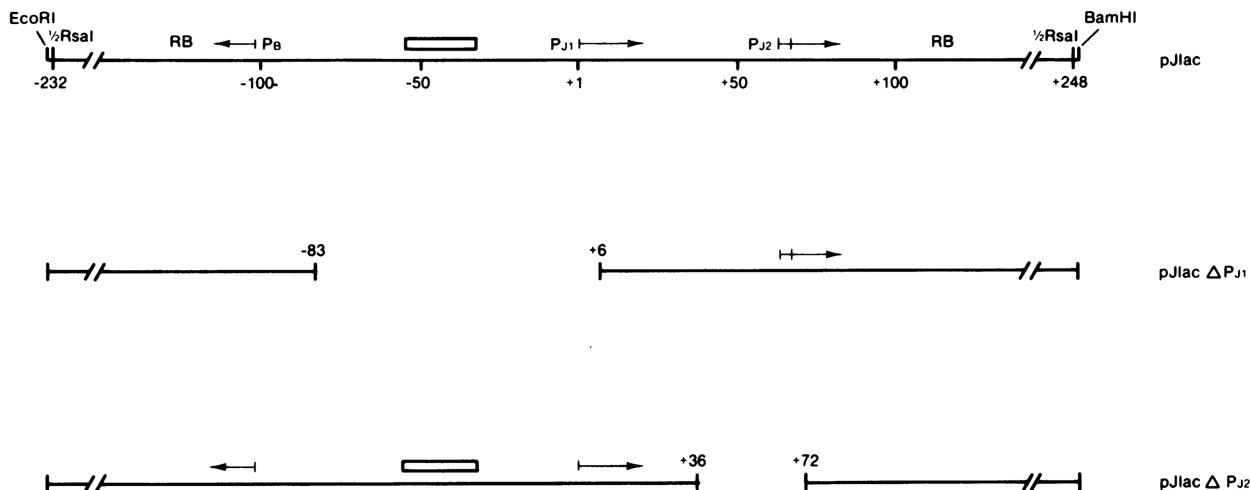


FIG. 2. Structure of the 490-bp *Eco*RI-*Bam*HI fragment of pJlac and two deletion derivatives, pJlac$\Delta P_{J1}$ and pJlac$\Delta P_{J2}$. The method of construction of the deletions is given in the text. Arrows indicate the start sites and direction of transcription from fragment promoters as determined by S1 nuclease mapping experiments (19; and see Results). A region proposed as a possible operator site for the *metB* gene in *E. coli* (14) is boxed. Sequence numbers correspond to those in reference 19, with +1 the transcription initiation site for promoter $p_{J1}$. $p_B$, *metB* promoter; $P_{J1}$ and $P_{J2}$, *metJ* promoters; RB, ribosome-binding site.

of Weaver and Weissmann (21) was used with modification as previously described (19). Essentially, the *Eco*RI-*Bam*HI fragment carrying the *metB* and *metJ* control regions from plasmid pJlac$\Delta P_{J1}$ or pJlac$\Delta P_{J2}$ was labeled at the *Bam*HI termini with $^{32}P$, the strands were separated, and the strand complementary to *metJ* mRNA from each was hybridized to total cellular RNA isolated from either GS243(pJlac$\Delta P_{J1}$) or GS243(pJlac$\Delta P_{J2}$), respectively, grown in glucose minimal medium plus D-methionine. Hybridization mixtures were then treated with S1 nuclease, and the S1 nuclease-resistant DNA products were electrophoresed alongside a sequencing ladder of the original DNA strand.

**Enzyme assays.** β-galactosidase activity was assayed as described by Miller (8), using the chloroform-sodium dodecyl sulfate lysis procedure.

**Chemicals and enzymes.** All chemicals and enzymes used are commercially available. The lambda-packaging system was from Amersham Corp., Arlington Heights, Ill.

## RESULTS

**Autoregulation of the *metJ* gene.** To test if the *metJ* gene product is involved in the control of the *metJ* gene itself, the λJlac phage was used to lysogenize a *metJ*$^+$ strain (GS243) and a *metJ* strain (GS597). The lysogens were then grown under conditions of methionine limitation and methionine excess, and the levels of β-galactosidase were measured. Expression of the fused *metJ-lacZ* gene is sensitive to repression by methionine in GS243(λJlac) (Table 2). In contrast, expression is elevated and nonrepressible by methionine in GS597(λJlac). These results suggest that the *metJ* gene product functions as a control element in its own regulation.

To be certain that the observed differences in β-galactosidase levels reflect gene expression mediated by the *metJ* gene product, we used λBlac lysogens as positive controls. Expression of the *metB-lacZ* gene is sensitive to repression by methionine in GS243(λBlac), but expression is elevated and nonrepressible by methionine in GS597 (λBlac)(Table 2).

**Activity of promoters $p_{J1}$ and $p_{J2}$.** Previous studies suggested that transcription of the *metJ* gene in *S. typhimurium* originates from two distinct start sites approximately 65 bp apart (19). It was proposed that two promoters, $p_{J1}$ and $p_{J2}$, exist for the *metJ* gene, although the possibility remained that the shorter transcript is a processed form of the longer transcript. To test the capability of both $p_{J1}$ and $p_{J2}$ to function as promoters, deletions were constructed in plasmid pJlac which inactivated either promoter $p_{J1}$ (pJlac$\Delta P_{J1}$) or $p_{J2}$ (pJlac$\Delta P_{J2}$). Figure 2 shows the extent of the deletions in relation to the *metJ* gene control elements. The deletion in pJlac$\Delta P_{J1}$ includes not only the proposed −10 and −35 regions of promoter $p_{J1}$, but also the sequence proposed as a possible operator site for the *metB* gene in *E. coli*. The

TABLE 2. Comparison of β-galactosidase levels of various *metJ-lacZ* fusion lysogens

| Strain | Promoter(s) being measured | β-Galactosidase sp act[a] | |
|---|---|---|---|
| | | L-Methionine | D-Methionine |
| GS243 (*metE*) | | <0.1 | <0.1 |
| GS597 (*metJ*) | | <0.1 | <0.1 |
| GS243(λJlac) | $p_{J1}$ + $p_{J2}$ | 79 | 145 |
| GS597(λJlac) | $p_{J1}$ + $p_{J2}$ | 278 | 278 |
| GS243(λJlac$\Delta P_{J1}$) | $p_{J2}$ | 23 | 24 |
| GS597(λJlac$\Delta P_{J1}$) | $p_{J2}$ | 21 | 23 |
| GS243(λJlac$\Delta P_{J2}$) | $p_{J1}$ | 7.5 | 33 |
| GS597(λJlac$\Delta P_{J2}$) | $p_{J1}$ | 81 | 79 |
| GS243(λBlac) | $p_B$ | 205 | 2,368 |
| GS597(λBlac) | $p_B$ | 7,529 | 7,634 |
| GS243(λBlac$\Delta P_{J2}$) | $p_B$ | 201 | 1,833 |

[a] Units of specific activity are nanomoles of *O*-nitrophenol produced per minute per milligram of protein at 28°C. For comparison, a fully induced culture that contains the wild-type *lac* operon and grown on glucose has about 1,000 U of activity (8). Growth medium was glucose minimal supplemented with phenylalanine, vitamin $B_1$, and either D-methionine (methionine limitation) or L-methionine (methionine excess).

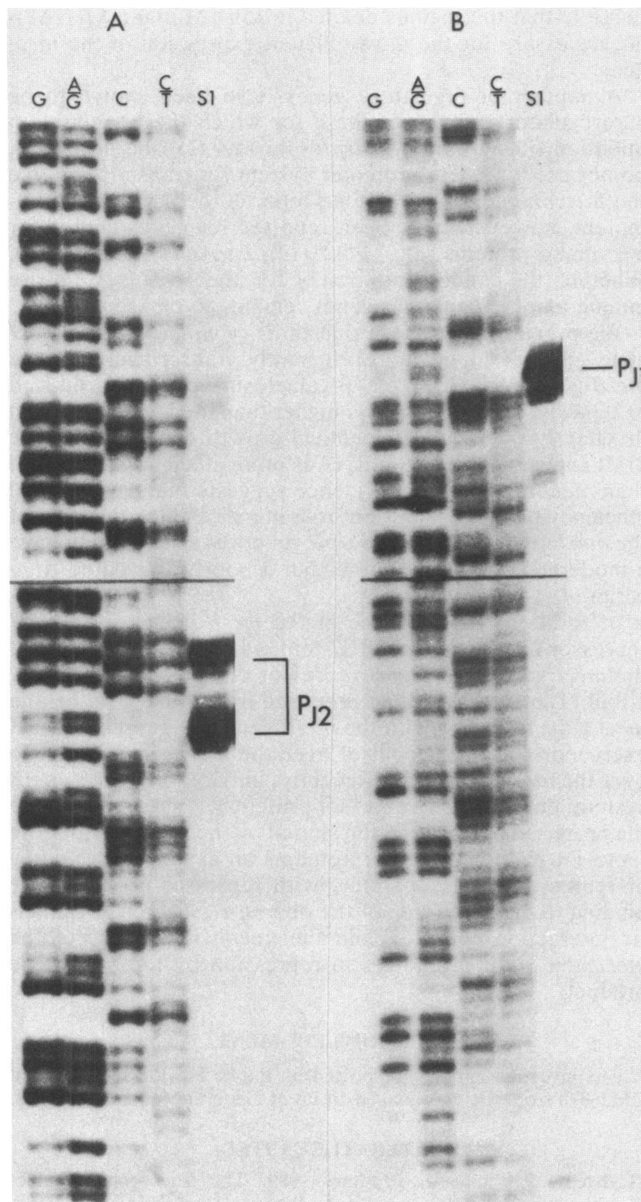


FIG. 3. Location of the 5′ termini of transcripts produced by (A) pJlacΔ$P_{J1}$ and (B) pJlacΔ$P_{J2}$. $P_{J1}$ and $P_{J2}$ indicate bases at which transcription initiation normally occurs in plasmid pGS107 (19). The first four lanes in each autoradiogram are the sequencing reaction products of the DNA strand used in the S1 nuclease hybridizations. Sequence-specific reactions are given at the top of each lane: S1 indicates the lane in which the S1 nuclease-protected products are run.

deletion in pJlacΔ$P_{J2}$ includes the proposed −10 region of $p_{J2}$, leaving $p_{J1}$ and the proposed operator sequence intact.

S1 nuclease mapping of the 5′ termini of the transcripts produced from these deletion plasmids indicates that transcription initiates at the expected transcription start sites for $p_{J1}$ and $p_{J2}$ (Fig. 3). This suggests that both $p_{J1}$ and $p_{J2}$ function as promoters in vivo and that the shorter transcript previously observed (19) results from transcription initiation at $p_{J2}$ rather than RNA processing. Furthermore, when the lambda derivatives of pJlacΔ$P_{J1}$ and pJlacΔ$P_{J2}$ (λJlacΔ$P_{J1}$ and λJlacΔ$P_{J2}$, respectively) are used to lysogenize the *lac* deletion strain GS243, both lysogens produce significant levels of β-galactosidase (Table 2).

To test whether promoters $p_{J1}$ and $p_{J2}$ repond to methionine repression mediated by the *metJ* repressor protein, strains GS243 (*metJ*$^+$) and GS597 (*metJ*) were each lysogenized with λJlacΔ$P_{J1}$ and λJlacΔ$P_{J2}$, and the β-galactosidase levels were measured in the lysogens grown under conditions of methionine limitation and methionine excess. β-Galactosidase activity in the *metJ*$^+$ lysogen GS243(λJlacΔ$P_{J2}$) is sensitive to repression by methionine, exhibiting about a 4-fold range of control, whereas in the *metJ* lysogen GS597(λJlacΔ$P_{J2}$) the enzyme levels are elevated an additional 2.5-fold and are non-repressible by methionine (Table 2). These results suggest that promoter $p_{J1}$ is under *metJ* repressor control. In contrast, GS243 (λJlacΔ$P_{J1}$) shows no difference in β-galactosidase levels between repressing and derepressing growth conditions, and the levels are not further elevated in GS597(λJlacΔ$P_{J1}$) (Table 2).

We used λBlacΔ$P_{J2}$ phage as a control to verify that the operator site had not been removed when promoter $p_{J2}$ was deleted. β-Galactosidase activity in the *metJ*$^+$ lysogen GS243(λBlacΔ$P_{J2}$) is sensitive to repression by methionine, exhibiting a ninefold range of control (Table 2).

## DISCUSSION

We have constructed a fused gene consisting of the *S. typhimurium metJ* control region and the *E. coli lacZ* gene to study regulation of expression of the *metJ* gene. A lambda phage carrying the *S. typhimurium metJ-lacZ* fusion (λJlac) was used to lysogenize either a *metJ*$^+$ or a *metJ E. coli* strain and the lysogens grown under conditions of either methionine limitation or methionine excess. The β-galactosidase levels are responsive over a 1.8-fold range to methionine addition to the growth medium in the *metJ*$^+$ lysogen, but are unresponsive to methionine and elevated 3 to 4-fold in the *metJ* lysogen (Table 2). These results suggest that the *S. typhimurium metJ* gene is autoregulatory in *E. coli*. A fusion of the *E. coli metJ* control region and the *lacZ* gene was recently reported (14). In this system, the *E. coli metJ* gene appears to also be regulated by its own gene product, but repression was observed to be independent of methionine supplementation to the growth medium. The cells in the experiments with the *E. coli metJ-lacZ* lysogens were Met$^+$ and therefore not limited for methionine. However, we routinely see about a 1.4-fold repression by methionine supplementation with *S. typhimurium* λJlac lysogens even when the host is Met$^+$ and thus not limited for methionine (data not shown).

We previously proposed that *metJ* mRNA is transcribed from two distinct promoters, $p_{J1}$ and $p_{J2}$, separated by about 65 bp (19). Construction of the two deletion derivatives of plasmid pJlac (pJlacΔ$P_{J1}$ and pJlacΔ$P_{J2}$) allowed us to look at the two promoters separately. When promoters $p_{J1}$ and $p_{J2}$ were each tested for the ability to initiate transcription, both were found to produce their respective mRNA transcripts (Fig. 3). In addition, when the lambda phage carrying these deletion derivatives (λJlacΔ$P_{J1}$ and λJlacΔ$P_{J2}$) were used to lysogenize *E. coli* hosts, both derivatives produced β-galactosidase (Table 2). Thus, both $p_{J1}$ and $p_{J2}$ function as true promoters in vivo.

Promoter $p_{J1}$ is regulated by both the *metJ* gene product and methionine supplementation to the growth medium. When the *metJ*$^+$ lysogen GS243(λJlacΔ$P_{J2}$), in which only promoter $p_{J1}$ functions, is grown under conditions of methionine limitation, β-galactosidase levels increase four- to

fivefold above the levels found when the lysogen is grown under conditions of methionine excess (Table 2). Furthermore, when the host *metJ* gene product is inactivated, as in the *metJ* lysogen GS597(λJlacΔ$P_{J2}$), enzyme levels are increased another two- to threefold and are no longer repressible by methionine addition to the growth medium. Thus, in these experiments a >10-fold range of regulation is seen for $p_{J1}$ when comparing fully repressed and fully derepressed conditions. This 10-fold range of regulation for $p_{J1}$ alone is significantly greater than the 3- to 4-fold range of regulation seen when $p_{J1}$ and $p_{J2}$ act in combination in the parent λJlac, as discussed above.

Promoter $p_{J2}$ did not respond to either methionine supplementation to the growth medium or the status of the *metJ* gene of the host. It is difficult to draw any conclusions from these results regarding the regulatory mechanism for $p_{J2}$, since the deletion in λJlacΔ$P_{J1}$ removes both $p_{J1}$ and the proposed operator sequence (Fig. 2). Thus, several explanations of the inability of promoter $p_{J2}$ to respond to either methionine or the *metJ* status of the host are possible and indistinguishable at this time. (i) $p_{J2}$ may be normally regulated by methionine and the *metJ* repressor much like $p_{J1}$, but in λJlacΔ$P_{J1}$ the operator site is deleted. However, it seems unlikely that $p_{J2}$ operates in the same manner as $p_{J1}$ (i.e., repressed by methionine), since it must temper the 10-fold range of activity of λJlacΔ$P_{J2}$ to the 3- to 4-fold range seen in the parent λJlac. (ii) $p_{J2}$ may be a constitutively expressed promoter. However, the relative proportion of *metJ* mRNA initiating at $p_{J2}$ versus $p_{J1}$ has been shown to vary under conditions of methionine limitation versus methionine excess in the growth medium (19). (iii) Transcription initiation at $p_{J2}$ could be dependent on the amount of transcription initiating at $p_{J1}$, which has been shown here to respond to methionine and the *metJ* gene product. In support of this hypothesis, we have shown previously that the RNA polymerase binding site for $p_{J1}$, determined by DNase I "footprinting," overlaps not only the proposed operator sequence, but also the expected RNA polymerase binding site for $p_{J2}$ (19). Thus, transcription initiation at $p_{J2}$ may be inhibited by the binding of RNA polymerase at $p_{J1}$. Growth conditions which lead to formation of the operator-repressor complex would inhibit RNA polymerase binding at $p_{J1}$ and thus stimulate transcription from $p_{J2}$. We are attempting to construct mutations in promoter $p_{J1}$ which would leave the proposed operator sequence and its spacing from $p_{J2}$ intact to clarify the regulation of $p_{J2}$.

It is interesting that the sum of the β-galactosidase levels given in Table 2 for promoters $p_{J1}$ and $p_{J2}$, when measured individually for a given host and growth condition, do not add up to the level seen in the parent λJlac for that same host and growth condition. In fact, the levels seen with λJlac are 2.5- to 3-fold higher than the sum of the deletion derivatives. The difference is probably not due to multiple copies of λJlac, since the bacterial strains have been lysogenized with the three phage in eight separate experiments, and the results are identical. It would be very unlikely to consistently obtain multiple lysogens with the λJlac phage and yet not obtain multiple lysogens with λJlacΔ$P_{J1}$ and λJlacΔ$P_{J2}$. It is also unlikely that two or more copies of the *metJ-lacZ* fused gene have been inserted into the λgt2 chromosome during the construction of λJlac. Such a recombinant chromosome would have a size of 55 kbp and would show a greatly reduced plaque size (22). In addition, the β-galactosidase seen for the totally derepressed lysogen GS597(λJlac) is very similar to the level reported for the *E. coli metJ-lacZ* fusion under similar conditions (14). It is possible that the regions deleted in λJlacΔ$P_{J1}$ and λJlacΔ$P_{J2}$ are necessary for the most efficient expression of the *metJ* gene.

A number of regulatory genes have been shown to be autoregulatory. Three of these for which the transcription initiation sites are known, *trpR* (4), *lexA* (1), and *araC* (20), do not use a tandem promoter system for transcription like the *S. typhimurium metJ* gene reported here. Tandem promoters, however, have been reported for genes coding for enzymatic proteins, e.g., *glnA* (12), *carA* (11), and *gal* (9). Whether the tandem promoters for the *metJ* system are unique among regulatory genes remains to be seen.

We previously reported that in *S. typhimurium* the *metJ* gene appears to be more efficiently transcribed than the *metB* gene (19). However, β-galactosidase levels produced in λBlac lysogens are always higher than the levels produced in λJlac lysogens under identical growth conditions (Table 2). It appears that *metB* mRNA is more efficiently translated than *metJ* mRNA. This evidence suggests that translational efficiency plays an important role in maintaining the level of the *metJ* gene product. The *trpR* gene was also found to have a moderately active promoter but a poorly translated transcript (4).

In addition, the response of the *metB* gene to the *metJ* repressor covers a range of 37-fold, whereas the response of the *metJ* gene to the *metJ* repressor covers a range of only 3-fold (Table 2). Since the proposed operator region for the *metJ* gene is shared with the *metB* gene, the repressor must exert more stringent control over the *metB* promoter than over the *metJ* promoters. Recently, in the *E. coli metJ-metB* system, purified *metJ* repressor protein was shown to bind to the proposed operator region shared by these genes (15). The footprint pattern was interpreted as an asymmetric binding of repressor to the operator, with repressor preferentially binding to the *metB* side of the operator. Such an asymmetric interaction could explain the unequal response of the *metJ* and *metB* promoters to repression by the *metJ* gene product.

## ACKNOWLEDGMENT

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