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Regulation of In Vivo Transcription of the *Escherichia coli* K-12 *metJBLF* Gene Cluster

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We subcloned DNA of the intercistronic region between the divergently transcribed *metJ* and *metB* genes of *Escherichia coli* into the transcription-fusion vector pK01 and localized the *metB* and *metJ* promoters by deletion analysis. The plasmid-borne promoters of both genes were repressed by chromosomal *metJ*. In addition, S1 nuclease mapping of chromosomally derived mRNA from a derepressed strain revealed the start sites of transcription for *metBL*, *metF*, and *metJ*. The *metBL* and *metF* genes each had a single transcript which was repressed by *metJ*, while the *metJ* gene had three transcripts, of which the first was strongly repressed by *metJ*, the second was less strongly repressed, and the third was not repressed.

The *metJBLF* gene cluster of *Escherichia coli* has been cloned, and the nucleotide sequence of the entire region has been determined (5, 22, 23). The cluster is organized into three transcriptional units (12). The *metB* and *metL* genes are transcribed as an operon, which is directly followed by the *metF* transcriptional unit. The *metJ* gene is transcribed from the opposite strand, and the coding sequence starts 276 nucleotides from that of *metB* (Fig. 1A) (22). The *metJ* gene codes for a regulatory protein which, in combination with *S*-adenosylmethionine, represses the genes of the *met* regulon. The *metJ* gene product exists as a dimer with an M_r of 24,000 and has been shown to bind specifically, in vitro, to the region of DNA between *metJ* and *metBL* and to the DNA near the *metF* promoter, causing repression of all four genes (24, 26). In this paper, we report the transcription start sites for the three transcriptional units and the locations of the *metBL* and *metJ* promoters. A preliminary report of some of this work has appeared previously (T. Kirby, B. Hindenach, and R. Greene, Fed. Proc. **44**:1416, 1985).

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* K-12 strains used in this study were as follows: RG109 *metK86* (6), JJ100 Hfr *metB1 relA1* (9), JJ135 Hfr *metB1 relA1 metJ185* (12), GW2540 Δ*lacU169 rpsL* Φ(*metE'-lacZ*$^+$)*170 met-176*::Tn*5* (19), and N100 *recA13 galK2* (15). Plasmid pAA110 has the *metJ* and *metB* genes on a modified pBR322 (25). Plasmid pRCG161 carries the *metF* gene (24). pUC8 (16), pDR720 (21), and pDR750 (21) have been described previously.

Materials. Restriction endonucleases, T4 polynucleotide kinase, and the Klenow fragment of DNA polymerase were purchased from New England BioLabs, Inc., Beverly, Mass., and Bethesda Research Laboratories, Gaithersburg, Md. S1 nuclease was obtained from Pharmacia Fine Chemicals, Piscataway, N.J. D-[1-^{14}C]galactose was obtained from Amersham Corp., Arlington Heights, Ill. [γ-^{32}P]ATP was prepared by the enzymatic method (10).

General methods. Ligations and transformations were performed essentially as described by Maniatis et al. (13), with minor variations. Plasmids were transformed into strain GW2540, which bears a *metE'-lacZ* fusion and a *metJ* mutation. When GW2540 is plated on rich medium containing 5-bromo-3-chloro-2-indolyl-β-D-galactoside (X-gal), colonies are blue due to expression of β-galactosidase from the derepressed *metE* promoter. Transformants with *metJ*-bearing plasmids gave rise to white colonies because of repression of transcription of the fused operon. LB plates containing 50 μg of ampicillin per ml and 40 μg of X-gal per ml were used to test transformants for *metJ*-bearing plasmids. The structures of all plasmids were confirmed by restriction mapping after small-scale preparation of plasmid DNA (3).

Galactokinase assays were performed by a previously published procedure (1). Transformants were grown to an optical density at 550 nm of 1 in M56 medium (18) supplemented with 0.2% Casamino Acids (Difco Laboratories, Detroit, Mich.) and 0.5% glucose, and cell extracts were prepared by sonication as described previously (1). Protein was assayed by a microbiuret procedure (28).

Subcloning *metJB*. A *Hae*III digest of plasmid pAA110 was separated on 1% agarose in TAE buffer (13). The 663-base-pair fragment, which contained the *metJ* gene and first 16 nucleotides of the *metB* coding sequence, was electrophoresed onto DEAE paper, eluted, and used to construct plasmids pKmJ and pKmB.

Construction of pKmJ. The *Hae*III fragment was ligated into the *Hin*cII site in the polylinker of pUC8 to yield pTK251. pTK251 was digested with *Eco*RI and *Hin*dIII, and the resultant 693-base-pair *metJ*-containing fragment was ligated into similarly cleaved pK01, to yield pKmJ. In this plasmid, galactokinase is transcribed from the *metJ* promoter(s).

Construction of pKmB. The *Hae*III fragment was ligated into the *Sma*I site of pDR720 to yield pTK201, allowing *metJ* to be transcribed from the *trp* promoter. A 720-base-pair *Eco*RI fragment of pTK201, containing the *Hae*III fragment and the *trp* promoter, was cloned into the *Eco*RI site of pDR750, and then a *Bam*HI fragment containing the *trp* promoter was deleted to form pTK222Δ. pTK222Δ was digested with *Eco*RI and *Hin*dIII, and the resultant 693-base-pair fragment was ligated into pK01 to finally yield pKmB. In this plasmid, galactokinase is transcribed from the *metB* promoter.

Deletions of pKmJ and pKmB. Plasmid DNA was digested with two appropriate restriction endonucleases, the product was treated with the Klenow fragment of DNA polymerase,

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and the resultant blunt-ended fragment was ligated to effect deletion of the sequence between the two restriction sites. The restriction sites used and the lengths of DNA deleted were as follows (see Fig. 2 for diagrams): pKmBΔES, *Eco*RI to *Sph*I, 185 base pairs; pKmBΔEN, *Eco*RI to *Nsi*I, 511 base pairs; pKmBΔSN, *Sal*I to *Nsi*I, 168 base pairs; pKmBΔSNΔES, *Eco*RI to *Sph*I, 185 base pairs, and *Sal*I to *Nsi*I, 168 base pairs; pKmJΔHS, *Hin*dIII to *Sph*I, 194 base pairs; pKmJΔHN, *Hin*dIII to *Nsi*I, 520 base pairs; pKmJΔBN, *Bam*HI to *Nsi*I, 164 base pairs; pKmJΔBNΔHS, *Bam*HI to *Nsi*I, 164 base pairs, and *Hin*dIII to *Sph*I, 194 base pairs.

S1 nuclease mapping. The labeled restriction fragments used for S1 nuclease mapping of *metJ* and *metB* were obtained from pKmBΔES or pTK251 by endonuclease digestion, followed by 5′ end-labeling with [γ-^{32}P]ATP and T4 polynucleotide kinase (14). The labeled fragments were then digested by a second restriction endonuclease and extracted with phenol.

RNA was prepared by the hot phenol method, as described by Aiba et al. (2). RNA (50 μg) was ethanol precipitated along with the 5′-end-labeled restriction fragment, and the dried pellet was dissolved in 20 μl of hybridization buffer [80% formamide, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.5), 0.4 M NaCl]. The mixture was incubated at 80°C for 10 min and then transferred to a water bath at 52°C (*metB* transcripts) or 47°C (*metJ* transcripts) for 2 to 15 h. Then, 155 μl of ice-cold water, 20 μl of 10× S1 nuclease buffer (0.3 M sodium acetate [pH 4.5], 0.5 M NaCl, 10 mM $ZnSO_4$, 50% glycerol), and 5μl of a solution of 5 U of S1 nuclease per μl were added; the mixture was kept at 30°C for 30 min and then precipitated with ethanol. The pellet was dissolved in loading solution (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.025% bromphenol blue, 0.025% xylene cyanol), heated to 85°C for 2 min, and electrophoresed on 6 or 4% polyacrylamide sequencing gels.

RESULTS

The sites of transcription initiation in the *metJBLF* gene cluster were identified in two ways. The transcription-fusion vector pK01 (15) was used to test for the presence of functional promoters on cloned segments of DNA, and the 5′ ends of transcripts were mapped with S1 nuclease (2).

Fusion of promoters to *galK*. Because *metBL* and *metJ* are divergently transcribed, promoters for both of them are likely to be found between the coding sequences of the two genes. A 663-base-pair *Hae*III fragment containing the entire *metJ* coding sequence and the first 16 nucleotides of the *metB* coding sequence, separated by a 276-base-pair intercistronic region, was cloned into the transcription-fusion vector pK01, in both orientations, giving plasmids pKmJ and pKmB. The structure of the inserted segment is diagrammed in Fig. 1. When the *galK* strain N100 was transformed with pK01, the transformants could not use galactose as the sole carbon source because there was very little transcription of the plasmid-borne copies of *galK*. Transformants of pKmB and pKmJ, however, were able to grow on galactose, indicating that transcripts can run off either end of the *Hae*III insert. Transformants also required methionine for growth, presumably because of the presence of multiple plasmid-borne copies of the *metJ* gene and consequent overproduction of the repressor; therefore, the cloned fragment contained all the sequences necessary for effective expression of the *metJ* gene.

The presence of several unique restriction sites in the plasmids, including a *Sph*I site in the *metJ* coding region and

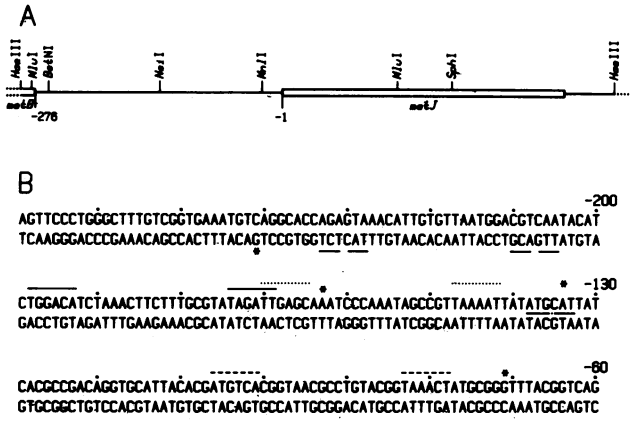
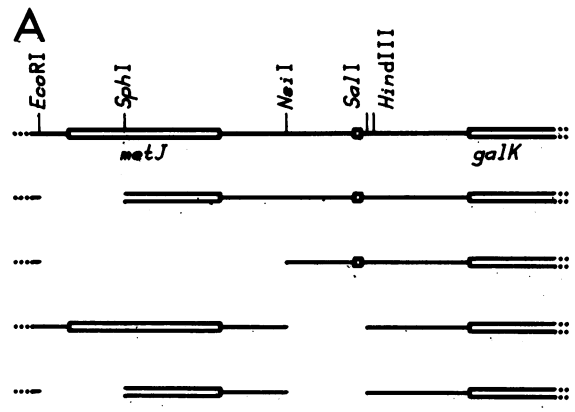


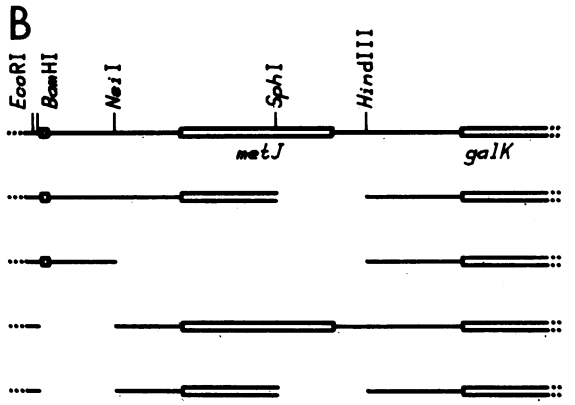
FIG. 1. (A) The 663-base-pair *Hae*III fragment from the *met* region of the *E. coli* chromosome. The fragment includes all of the *metJ* coding region and 16 nucleotides of the *metB* coding region. Relevant restriction endonuclease cleavage sites are shown. (B) Nucleotide sequence of part of the intercistronic region between *metJ* and *metB*, from Saint-Girons et al. (22). The first nucleotide of the *metJ* coding sequence is 1, as defined by Shoeman et al. (23a). The −35 and −10 regions of the three *metJ* promoters are designated by the lines above the top strands (——, J1; ....., J2; - - - - -, J3), and the −35 and −10 regions of the *metB* promoter are designated by the broken line below the bottom strand. The asterisks indicate the transcriptional start sites. The recognition site for restriction endonuclease *Nsi*I has a broken line between the strands.

a *Nsi*I site in the middle of the intercistronic region, allowed convenient analysis of *metJ* and *metB* promoters by deletion of various segments of *met* DNA in pKmJ and pKmB. Four deletion derivatives of each plasmid were constructed as described above (Fig. 2). In the first set of derivatives, pKmBΔES and pKmJΔHS, the *metJ* gene was inactivated by deletion of DNA between the *Sph*I site and an appropriate restriction site of the vector, which removes the carboxy-terminal portion of the *metJ* coding sequence. In the second set, pKmBΔEN and pKmJΔHN, the entire *metJ* coding region and half of the intercistronic region, up to the *Nsi*I site, was removed. In the third set, pKmBΔSN and pKmJΔBN, the other half of the intercistronic region and the *metB* coding region were deleted, but the *metJ* coding sequence was left intact. In the fourth set, pKmBΔSNΔES and pKmJΔBNΔHS, the DNA after the *Sph*I site was removed from the third set of plasmids, thereby inactivating *metJ* as before.

Analysis of the *metB* promoter. Plasmids pKmB and its four deletion derivatives were used to transform the *galK* strain N100. Transformants with plasmids pKmB, pKmBΔES, and pKmBΔEN grew on galactose, while those with plasmids pKmBΔSN and pKmBΔSNΔES did not grow, indicating that the region deleted in pKmBΔSN contains the *metB* promoter. Strains JJ100 and its *metJ* derivative JJ135 were also transformed with the plasmids, and extracts of the transformants were assayed for galactokinase to determine the effect of chromosomal *metJ*. The transformants were grown on medium containing glucose and lacking galactose to repress the chromosomal copy of *galK*. Results of the assays are shown in Fig. 2A. In both backgrounds the *metB* promoter from pKmB directed only a low level of *galK* expression because it was repressed by the product of the plasmid-borne *metJ* gene. Inactivation of the *metJ* gene in pKmBΔES and pKmBΔEN resulted in slight derepression of the JJ100 transformants and marked derepression of the



PLASMID		HOST STRAIN JJ100 (metJ⁺)	HOST STRAIN JJ135 (metJ⁻)
		GALACTOKINASE (units/mg)	
pKmB	(*metJ*⁺)	75	56
pKmB Δ ES	(*metJ*⁻)	151	4684
pKmB Δ EN	(*metJ*⁻)	164	5802
pKmB Δ SN	(*metJ*⁺)	28	31
pKmB Δ ES Δ SN	(*metJ*⁻)	24	30



PLASMID		HOST STRAIN JJ100 (metJ⁺)	HOST STRAIN JJ135 (metJ⁻)
		GALACTOKINASE (units/mg)	
pKmJ	(*metJ*⁺)	530	461
pKmJ Δ HS	(*metJ*⁻)	597	1225
pKmJ Δ HN	(*metJ*⁻)	217	706
pKmJ Δ BN	(*metJ*⁺)	313	200
pKmJ Δ HS Δ BN	(*metJ*⁻)	130	108

FIG. 2. (Left) Deletion plasmids. A blank space indicates the region that was deleted. Plasmids were constructed as described in the text. (Right) Galactokinase transcription from the deletion plasmids. Transformants of *E. coli* JJ100 (*metJ*⁺) and JJ135 (*metJ*⁻) were assayed for galactokinase. Units are expressed as nanomoles of galactose-phosphate formed per minute at 37°C. (A) pKmB derivatives; (B) pKmJ derivatives.

JJ135 transformants. Thus, the single chromosomal copy of *metJ* in JJ100 produces enough product to cause about a 35-fold repression of the multiple, plasmid-borne *metB* promoters. Also, it is clear that the *metB* promoter is located between the *Nsi*I site and the *metB* coding region, because deletion of this DNA in pKmBΔSN and pKmBΔSNΔES reduced galactokinase activity to approximately that of pK01. Furthermore, the low level of *galK* expression from pKmBΔSN and pKmBΔSNΔES is not subject to repression by *metJ*.

Analysis of the *metJ* promoters. Plasmids pKmJ and its four deletion plasmids were also used to transform strain N100. All transformants were capable of growth on galactose, indicating that *metJ* has multiple promoters. Galactokinase assays on JJ100 and JJ135 transformants (Fig. 2B) showed that the *metJ* promoters of pKmJ appear to be about eightfold more active than the *metB* promoter of pKmB. Both plasmids directed enough synthesis of the *metJ* gene product to greatly repress the *met* regulon and thus impose a methionine requirement on their host strain. Inactivation of *metJ* on plasmid pKmJΔHS did not affect *galK* expression in JJ100, but it did in JJ135; the complete absence of functional *metJ* effected a two- to threefold increase in galactokinase activity. Deletion of all of the *metJ* coding region and half of the intercistronic region (pKmJΔHN) lowered the *galK* expression, but the remaining activity was still subject to repression by chromosomal *metJ*. Deletion of the other half of the intercistronic region (pKmJΔBN) resulted in lower expression of *galK* which was not repressed by chroı o-somal *metJ*. While pKmJΔBN does express *metJ*, as evidenced by its ability to repress the *metE'-lacZ* fusion of GW2540, the expression is not great enough to cause a methionine requirement in transformants. When the *metJ* gene on pKmJΔBN was inactivated, forming pKmJΔBNΔHS, the expression of *galK* decreased slightly, and again, chromosomal *metJ* had little effect. In summary, the results indicate that *metJ* promoters exist on either side of the *Nsi*I site and that the autoregulation of *metJ*, which has been demonstrated by Saint-Girons et al. (22) for a fusion protein, appears to involve the longer transcript(s).

Mapping the 5' end of the *metB* transcript. The S1 nuclease mapping procedure was used to determine the 5' end of *metB* mRNA. Figure 3 shows the portion of the *Mlu*I-*Mnl*I fragment protected from S1 nuclease digestion by hybridization to the *metB* transcript present in RNA prepared from the *metJ* strain JJ135 (Fig. 3, lane 3). No transcript was detected in RNA from the *metJ*⁺ strain JJ100 (Fig. 3, lane 2) or JJ135 harboring plasmid pKmB (Fig. 3, lane 4). An experiment using various concentrations of S1 nuclease showed that the lower bands in Fig. 3, lane 3, are artifacts of the procedure. The initiation site for the chromosomal *metB* transcript is nucleotide −244 (Fig. 1B). The *metB* transcript originating from plasmid pKmBΔES also starts at nucleotide −244 (Fig. 1B; data not shown). The *met* DNA in this plasmid originally

came from a strain that is wild type for *metB*, indicating that the *metB1* mutation in JJ135 is not responsible for an anomalous transcript. A computer search of the intercistronic region for consensus promoter sequences by the method of Mulligan et al. (20) revealed a good promoter, with a score of 58.0, in the correct position to direct the synthesis of the observed *metB* transcript.

Mapping of the 5′ ends of *metJ* transcripts. S1 nuclease mapping, using a *Bst*NI-*Mlu*I fragment 5′ end labeled at the *Mlu*I site and hybridized to RNA isolated from various sources (Fig. 4), revealed that there are three *metJ* transcripts. The transcripts are referred to as J1, J2, and J3, and their start sites are nucleotides −166, −137, and −74, respectively (Fig. 1B). The presence of a corresponding consensus promoter sequence for each of the three transcripts leads us to believe that all three may be primary transcripts. Figure 4, lane 2, shows that only the J2 and J3 transcripts are present in JJ100, while lane 3 shows that JJ135 has all three transcripts (both strains were grown in rich medium). Thus, the functional copy of *metJ* in JJ100

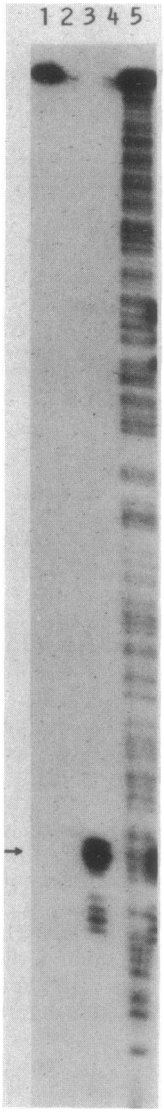


FIG. 3. S1 nuclease mapping of chromosomal *metB* transcripts. S1 nuclease mapping was performed by standard procedures using, as the DNA probe, a 261-base-pair *Mlu*I-*Mnl*I fragment of pKmBΔES 5′ end-labeled at the *Mlu*I site. The DNA was hybridized with RNA from several sources. Lane 1 has no S1 nuclease; lane 2, JJ100; lane 3, JJ135; lane 4, JJ135 transformed with pKmB. Lane 5 shows the A+G sequencing reaction of the fragment. The samples were electrophoresed on a 6% polyacrylamide sequencing gel. Arrow, Position of the fragment protected by the *metB* transcript.

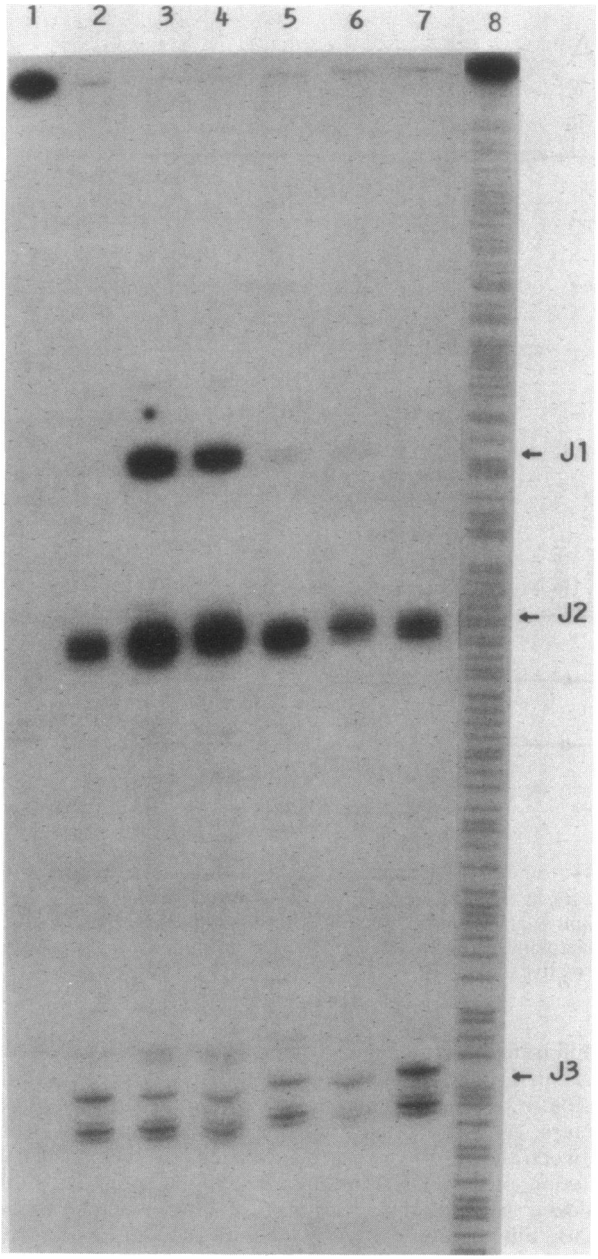


FIG. 4. S1 nuclease mapping of chromosomal *metJ* transcripts. S1 nuclease mapping was performed by standard procedures using, as the DNA probe, a 389-base-pair *Mlu*I-*Bst*NI fragment of pKmBΔES 5′ end-labeled at the *Mlu*I site. The DNA was hybridized with RNA from several sources. Lane 1, no S1 nuclease; lane 2, JJ100; lane 3, JJ135; lane 4, RG109 grown on minimal medium; lane 5, RG109 grown on LB; lane 6, strain K-12 grown on minimal medium; lane 7, strain K-12 grown on LB. Lane 8 shows the A+G sequencing reaction of the fragment. The samples were electrophoresed on a 4% polyacrylamide sequencing gel.

very effectively represses transcription from the first *metJ* promoter. Because strain JJ135 has an amber mutation in *metJ*, the possibility arose that the J1 transcript may not be found in cells with a functional *metJ* gene. Therefore, the *metJ* transcripts were examined in two strains with functional *metJ*: a *metK* strain and wild-type prototrophic *E. coli* K-12. It has been shown that a mutation in the *metK* gene leads to derepression of the *met* regulon, presumably because the *metJ* gene product and *S*-adenosylmethionine, the product of methionine adenosyltransferase (*metK* gene product), are both required for repression (6). Figure 4, lane 4, shows that RNA from the *metK* strain RG109 contains all three transcripts when the cells are grown in minimal medium, i.e., under fully derepressing conditions. Growth of the *metK* strain in rich medium leads to some repression, and lane 5 shows that the J1 transcript is reduced. Wild-type *E. coli* K-12, grown in minimal medium, was also derepressed, albeit not as much as a *metK* mutant, and Fig. 4, lane 6, shows that there is a small amount of the J1 transcript in these cells. Again, rich medium lead to the elimination of that transcript (Fig. 4, lane 7). The J2 and J3 transcripts appeared to be present in all cells. Figure 4 also indicates that in repressed cells the J2 transcript appears to be reduced and there may be some slight increase in the J3 transcript.

Mapping of the 5′ end of the *metF* transcript. Saint-Girons et al. (23) have mapped the 5′ end of the *metF* transcript in the *metJ* strain Gif 881L. Using the *metF*-bearing plasmid pRCG161, Shoeman et al. (24) have shown in vitro expression of *metF*, which is repressed by the *metJ* gene product and *S*-adenosylmethionine. We used the S1 nuclease mapping procedure with a suitable, labeled probe from pRCG161 to characterize the *metF* transcript in three plasmid-free strains and in two pRCG161 transformants (data not shown). In the derepressed strains, RG109 (*metK*), JJ135 (*metJ*), and the pRCG161 transformant of JJ135, a single *metF* transcript was observed. This transcript appears to be the same as that described by Saint-Girons et al. (23), starting about 68 nucleotides before the coding sequence. In the repressed strains, JJ100 and its pRCG161 transformant, no *metF* message was detectable, showing that transcription of both the chromosomal and plasmid-borne *metF* gene is blocked by the *metJ-metK* regulatory system.

DISCUSSION

We located the start sites for transcription of the *metB*, *metJ*, and *metF* genes of *E. coli* K-12 and identified four promoters in the 276-base-pair intercistronic region between the *metB* and *metJ* coding sequences. Our results for the *metB* transcript disagree with those of Duchange et al. (5), who have reported the location of the transcription start site of the cloned *metB* gene of pMAD4. We dissected the intercistronic region into *metB* proximal and distal segments and subcloned them into the promoter detection vector pK01. The *galK* expression from the derivative carrying the distal segment, which contained the purported promoter sequence, was no greater than that from pK01 alone. However, the *metB* proximal segment, in pKmBΔEN, directed a high level of *galK* expression in a *metJ*$^-$ strain (Fig. 2A). The expression was strongly repressed in a *metJ*$^+$ strain, showing that this promoter is subject to the *met* regulatory system. When we searched the intercistronic region for consensus promoter sequences (20), we found the sequence with the best score (58) to lie within the segment which gave controlled expression of *galK*. In contrast, the purported promoter gave a score of 41, which is below the suggested cutoff value of Mulligan et al. (20).

When we examined the mRNA of a derepressed, plasmid-free strain, we detected a single *metB* transcript which was 97 nucleotides shorter than that reported by Duchange et al. (5). This transcript was not detectable in repressed cells, showing that it is subject to the *metJ*-mediated control system. The 5′ end of the *metB* mRNA is located near the high-scoring promoter sequence (Fig. 1), so it is most likely a primary transcript. In addition, the site that we found for initiation of *metB* transcription is the same as that found by Urbanowski and Stauffer for the *metB* gene of *Salmonella typhimurium* (27). While the reason for the discrepancy between our results and those of Duchange et al. is not clear, it should be noted that they assayed RNA from a strain transformed with a multicopy plasmid bearing functional *metJ*. When we performed a similar experiment (using a pKmB [*metJ*$^+$] transformant), we did not find any *metB* transcripts when normal gel exposure times were used. This result was expected, because such plasmids cause the overproduction of the *metJ* product, effecting repression of *met* gene expression (25). However, after prolonged exposure of the S1 nuclease assay gels, we did detect a very faint band near the position of the transcript of Duchange et al. The origin of this RNA is obscure; perhaps it is a very weakly transcribed mRNA or a point of RNA processing for a low level of upstream plasmid-directed transcription.

In contrast to *metB*, which has a single promoter, the *metJ* gene appears to have three promoters and three transcripts. The J1 transcript is most prominent in cells with *metJ* or *metK* mutations (i.e., derepressed cells), although a trace of it is present in wild-type *E. coli* K-12 grown on minimal medium. Transcription from the J1 promoter was very effectively inhibited by repressing conditions. In cells with a fully functional *met* regulatory system, it appeared that transcription from the J2 promoter was also reduced, although not nearly to the same extent as that from J1, and that the J3 transcript was not repressed at all.

In their analysis of *metJ* transcription in *S. typhimurium*, Urbanowski and Stauffer (27) found only two transcripts, which would correspond to our J2 and J3. Because the promoter sequence we designated J1 is conserved in *E. coli* and *S. typhimurium*, it probably functions in both organisms. All of the work done with *S. typhimurium* was with strains that had a functional *met* control system, so it is likely that the first promoter was repressed. Urbanowski and Stauffer (27) also suggested that transcription from the *S. typhimurium* promoter corresponding to our J3 may be stimulated by *metJ* protein binding. Our results are in agreement, but the effect is small. Thus, the wild-type K-12 strain had a relatively larger amount of the J3 transcript when grown on LB medium than when grown on minimal medium. In addition, when the *metJ* strain JJ135 was transformed with pKmJΔBN, it directed synthesis of twice as much galactokinase as when it was transformed with pKmJΔBNΔHS, the major difference being that the latter plasmid lacks a functional *metJ* gene (both plasmids have *galK* transcribed only from the J3 promoter). Regardless of the basis of the apparent stimulatory effects, it is clear that the *metJ* protein is not required for transcription from the J3 promoter, because the J3 transcript is present in cells that completely lack *metJ*.

We have previously shown (26) that the *metJ* protein binds to DNA of the intercistronic region, protecting 40 to 50 base pairs from digestion by DNase I. The −35 sequence of the *metB* promoter, the entire J1 promoter, and possibly part of the −35 sequence of the J2 promoter lie within the protected region. Thus, the in vitro binding of the *metJ* protein is

consistent with our observations on in vivo transcription of the two genes. It appears that binding of the *metJ* gene product may prevent binding of RNA polymerase to the *metB* and J1 promoters and, under strongly repressing conditions, may also decrease the binding to the J2 promoter. It should also be noted that the *metB* promoter proposed by Duchange et al. (5) lies outside of the in vitro *metJ* protein binding region.

A comparison of the published *E. coli* K-12 (22) and *S. typhimurium* (27) *metJB* nucleotide sequences revealed several regions which are highly conserved. The *metJ* coding sequences (318 nucleotides) are 94% homologous. The intercistronic regions differ in length (276 nucleotides in *E. coli* versus 264 nucleotides in *S. typhimurium*) and have some segments of high homology interspersed with others of low homology. All −35 and −10 promoter sequences are conserved, except for two single nucleotide changes (one in the J3 promoter and one in the *metB* promoter) which do not greatly affect the homology of the promoters to the consensus sequence (20). It is interesting that the most conserved region, in which 80 of 82 nucleotides are identical, contains the in vitro binding site for the *metJ* gene product. Another segment of high homology occurs at the end of the intercistronic region in which 26 of the first 27 nucleotides preceding the *metJ* coding sequence are the same in both organisms; the significance of this homology is unknown.

While the 3′ end of the *E. coli metJ* transcript has not yet been determined, the end of the *S. typhimurium* transcript has been reported to be located 40 nucleotides from the translation termination codon of the *metJ* gene (27). The *Hae*III fragment employed in constructing pKmJ and pKmB includes 53 nucleotides following the *metJ* coding region, but it is apparent from our transcription-fusion data that that region (which is completely divergent from the *S. typhimurium* sequence) does not contain signal(s) sufficient to terminate the *metJ* transcript. The *metJ* promoters directed essentially equal levels of *galK* transcription in fusions both with and without the 53-nucleotide region.

The *metJ* gene has been added to the list of regulatory proteins which are autoregulated, based largely on results with *metJ-lacZ* fusion proteins in *E. coli* (22) and *S. typhimurium* (27). The regulation has been postulated to be at the level of transcription due to the presence of a suspicious region of dyad symmetry (the "*met* box") (22) and to the in vitro binding of the purified *metJ* gene product near the *met* box (26). Our results confirm that *metJ* is autoregulated and demonstrate that much of the regulation is indeed at the level of transcription (i.e., repression of the J1 and J2 promoters). The ratio of *metJ* expression in fully derepressed cells to that in repressed cells is between 3 and 7. This is comparable to the result with *trpR* (11), but the details of regulation are different for the two genes. *trpR* appears to have a single promoter which is weakly repressible by the *trpR* gene product (7). On the other hand, *metJ* has three promoters, of which the first is highly sensitive to repression, the second is partially repressible, and the third is constitutive.

Another difference between the repressors *metJ* and *trpR* concerns super repression. When the *metJ* gene is present on a multicopy plasmid, the intracellular concentration of the gene product appears to be greatly increased, as the plasmid confers a methionine requirement on cells. In contrast, multicopy plasmids containing the *trpR* gene do not cause such super repression (4). Because the maximum repressibility of our *metJ-galK* fusion was about the same as that of a *trpR-lacZ* operon fusion (11), the difference could be due to better translation of the *metJ* messages compared with the reportedly inefficient translation of the *trpR* message (11).

While we have not directly measured the amount of the *metJ* peptide in any of the strains, some results suggest that its concentration is relatively high compared with that of other regulatory proteins. The single chromosomal *metJ* gene produces enough protein to cause at least a 30-fold repression of the multiple copies of pKmBΔES or pKmBΔEN (Fig. 2A). In a number of regulatory systems, it has been shown that multiple copies of a cloned operator can sequester the chromosomal repressor and effect increased expression of the chromosomal genes, which are subject to the same regulatory elements. Such repressor titration in *trans* occurs for the *trp* (11) and the *gal* (8) operons in *E. coli*, but apparently not for the *met* regulon. The *metB-galK* fusion plasmid pKmBΔES bears the *metJ* binding site but does not carry a functional *metJ* gene, and yet the plasmid-directed galactokinase activity in a *metJ*$^+$ transformant, grown under repressing conditions, is largely repressed. In addition, we found no effect of the plasmid on expression of the chromosomal *metC* gene (data not shown), indicating that the plasmid did not cause a marked decrease in the amount of available *metJ* protein. The *metF* (this work) and *metA* (17) genes, when present on multicopy plasmids, have also been shown to be effectively repressed by the chromosomally derived pool of the *metJ* protein. All these results are consistent with our previous in vitro observations (26), which suggest that the *metJ* peptide has a relatively low affinity for the *metBL metJ* operator region. Thus, the concentration of *metJ* peptide would have to be relatively high to be functional and, consequently, the peptide would not be easily depleted.

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