JOURNAL OF BACTERIOLOGY, Feb. 1984, p. 413–419
0021-9193/84/020413-07$02.00/0

Vol. 157, No. 2

Physical Organization of the *metJB* Component of the *Escherichia coli* K-12 *metJBLF* Gene Cluster

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Received 19 July 1983/Accepted 3 November 1983

The structures of a series of plaque-forming *metJB* transducing phage were studied by restriction endonuclease mapping and enzyme activity assay. One of these phage, λ p*met*100, was inactivated by heat shock in the presence of EDTA, and deletion mutants were selected from the survivors. Two of these mutants, λ p*met*100Δ1 and λ p*met*100Δ2, were used to confirm the gene order *metJ metB* when moving clockwise on the linkage map of *Escherichia coli* K-12. Additional results indicate that the *metB* gene can be expressed independently of any other component of the *met* regulon and that the *metJ* gene also forms a separate transcription unit.

The order of the *metJB* component of the *metJBLF* gene cluster, which is located at 88 min on the linkage map of *Escherichia coli* K-12, has been reported in both orientations (2, 12, 28). Recent reports demonstrate that the cluster's transcriptional organization does not correspond to that of an operon (14, 21, 28). However, details of this organization are incomplete. The *metF* component of the gene cluster exists as a separate transcription unit (14), and the limits of the gene cluster have been defined to be 5.6 to 6.0 kilobase pairs (kbp) (6). The *metBLF* region has been cloned from the λ d*met*102/117 (10) and λ h80 d*met*A4 (23) transducing phage into high-copy-number plasmids, and a subclone of this region (5.0 kbp) containing the *metB* gene in an expressible form was constructed (28). However, the presence of the *metJ* gene and the transcriptional organization of the *metJB* component of the cluster were not addressed (28). The sequence of the *metL* gene has been determined, but a promoter was not identified (27). To study their gene order and transcriptional organization, plaque-forming lambda transducing phage which contain the *metJB* component of the cluster were constructed by this laboratory. This paper reports the characterization of these transducing phage and the results of deletion analysis of one isolate which confirm an earlier report (12) that the gene order is *metJBLF*. In addition, data are presented which demonstrate that *metJ* (which codes for the proposed *met* regulon aporepressor protein [9, 14, 20, 26]) and *metB* (which codes for the enzyme cystathionine-*gamma*-synthetase) can be expressed independently of other genetic elements of the *met* regulon and of each other.

(C.A.L. completed portions of this project in partial fulfillment of the requirements for the Ph.D. degree, Texas A&M University.)

MATERIALS AND METHODS

Strains. The *cytR* insertion lysogen CL135 (*attB gal bio thi* λ *c*I857 *S*am7) was the source of the plaque-forming *met* transducing phage. Figure 1 shows the construction of this lysogen. All other phage and bacterial strains used in this study are listed in Table 1.

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Media. All media used for enzyme assays, phage growth, and plaque assays have been described previously (10). EMBO plate medium was used in the construction of lysogens and is described by Miller (19).

Enzymes and chemicals. Restriction endonucleases were purchased from Bethesda Research Laboratories or New England BioLabs, except for *Eco*RI which was prepared by modifications of procedures described by Mayer and Reichenbach (16) and Baski et al. (3). All other chemicals were purchased from standard sources and were of reagent grade.

Isolation of plaque-forming transducing phage. A CL135 lysate was prepared and, to eliminate defective phage, a plate lysate was produced by using RW262 cells. The plate lysate was diluted and screened for plaque-forming *metB* transducing phage as described below (11). A fresh culture of JJ131F (*metJ184 metB1 supF* [$tRNA^{Tyr}$] [2]) (Table 1) was grown in Davis-Mingioli minimal salts medium (0.5% maltose, 0.2 mM methionine), washed, and resuspended in Davis-Mingioli minimal salts at an optical density at 550 nm of 0.80. Subsequently, 0.2 ml of the cell suspension and 0.1 ml of an appropriate dilution of the plate lysate were combined, and 20 μl of 20 mM L-methionine plus 2.5 ml of molten Davis-Mingioli minimal salts top agar were added. The resulting solution was poured onto Davis-Mingioli minimal maltose plates and allowed to harden at room temperature. These plates were incubated overnight at 42°C to allow plaque formation and for 24 h at 32°C to allow transduction and growth of cells in the bacterial lawn surrounding the formed plaques. The plaques produced by $metB^+$ transducing phage develop a haloed appearance (11). Haloed plaques were picked into sterile dilution buffer and stored over chloroform. Phage suspensions were tested by spot transduction on lawns of JJ100 (*metB1*), JJ122 (*metF*), and RG776 (*metL*) cells. Phage demonstrating transducing ability were used to construct lysogens of JJ116. Table 1 provides a partial list of the phage isolated during this study. Purified phage were prepared by described methods (13).

DNA preparation. DNA was prepared by disruption of purified phage with sodium dodecyl sulfate (0.4%) and extraction with equilibrated phenol followed by dialysis of the aqueous phase against appropriate buffers (13). DNA concentrations were determined by measuring the absorbance of the dialyzed material at 260 nm.

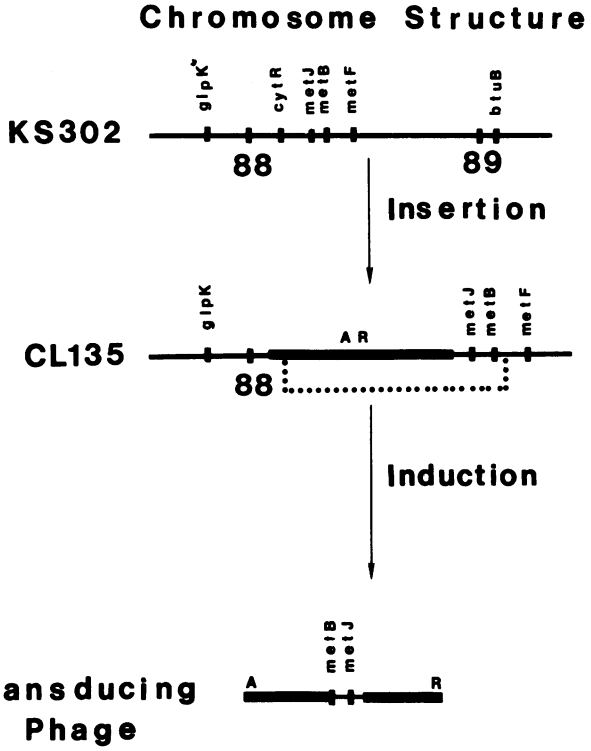
Nuclease digestion and electrophoresis of phage DNA. DNA

TABLE 1. Bacterial and bacteriophage strains

Strain	Relevant genotype	Source
Bacterial nonlysogens		
JJ116	Prototroph	Johnson et al. (10)
JJ119	*metJ185*(Am)	Johnson et al. (10)
JJ100	*metJ*$^+$ *metB1 metL*$^+$ *metF*$^+$	Krueger et al. (14)
JJ122	*metJ*$^+$ *metB*$^+$ *metL*$^+$ *metF*^{a}	Johnson et al. (10)
JJ131	*metJ184 metB1 metL*$^+$ *metF*$^+$	Johnson et al. (10)
RG776^b	*metJ metB*$^+$ *metL metF*$^+$ *thrA*	R. C. Greene
RW262	*tonA supF mal*	Mazaitis et al. (17)
JJ131F	*metJ184 metB1 metL*$^+$ *metF*$^+$ *supF*	Johnson and Liljestrand (11)
JJ100F	*metJ*$^+$ *metB1 metL*$^+$ *metF*$^+$ *supF*	Johnson and Liljestrand (11)
Bacterial lysogens		
JJ131(0)	JJ131 lysogen of λ p*met*100	This study
JJ131F(0)	JJ131F lysogen of λ p*met*100	This study
JJ131(0J5)	JJ131 lysogen of λ p*met*100J5	This study
JJ131F(0J5)	JJ131F lysogen of λ p*met*100J5	This study
JJ100F(0Δ1)	JJ100F lysogen of λ p*met*100Δ1	This study
JJ100F(0Δ2)	JJ100F lysogen of λ p*met*100Δ2	This study
JJ131F(0Δ1)	JJ131F lysogen of λ p*met*100Δ1	This study
JJ131F(0Δ2)	JJ131F lysogen of λ p*met*100Δ2	This study
JJ116(0)	JJ116 lysogen of λ p*met*100	This study
JJ116(0Δ1)	JJ116 lysogen of λ p*met*100Δ1	This study
JJ116(0Δ2)	JJ116 lysogen of λ p*met*100Δ2	This study
JJ119(0)	JJ119 lysogen of λ p*met*100	This study
JJ119(0Δ1)	JJ119 lysogen of λ p*met*100Δ1	This study
JJ119(0Δ2)	JJ119 lysogen of λ p*met*100Δ2	This study
Bacteriophage		
λ p*met*100	*metJ*$^+$ *metB*$^+$	This study
λ p*met*100J5	*metJ185*(Am)	This study
λ p*met*100Δ1	Δ(*metJ-metB*)	This study
λ p*met*100Δ2	Δ(*metJ*) *metB*$^+$	This study
λ p*met*101, -103	*metJ*$^+$ *metB*$^+$	This study
λ p*met*102	*metJ*$^+$ *metB*$^+$	This study

^{a} The *metF* allele is from strain RG348 and was obtained from R. C. Greene.

^{b} This strain is a derivative of Gif881 (*metJ metB*$^+$ *metL*$^+$ *thrA*) (28) and was isolated as a spontaneous mutant (R. C. Greene, personal communication).



molecules were digested with *Eco*RI, *Sma*I, *Hpa*I, *Sst*II, or *Pvu*I for 4 h at 37°C in the appropriate digestion buffer (as referenced in the 1982 Bethesda Research Laboratories or NewEngland BioLabs catalog). Reactions were stopped by addition of 20 mM EDTA (final concentration). DNA digests were electrophoresed, with minor modifications, according to described procedures (18). Migration distances of restriction fragments were measured from photographs of gels, and molecular weights were determined by interpolation from semilog plots of the molecular weights versus the relative migration distances of standards run on the same gel (8).

Enzyme assays. Cystathionine-*gamma*-synthetase (*metB*) was assayed in a two-part coupled assay (9). *Beta*-cystathionase (*metC*) was assayed by monitoring the cleavage of Ellman reagent with a recording spectrophotometer (9) or by a semiquantitative microscale assay (5). Protein concentrations were determined by the method of Lowry et al. (15).

EDTA/heat inactivations. Phage λ p*met*100 was diluted into

FIG. 1. Diagram of the events leading to the construction of the secondary attachment site lysogen, CL135, and to the formation of specialized plaque-forming transducing phage. The attachment site-deleted strain KS302 (25) was infected with λ *c*I857 *S*am7, and the secondary insertion lysogen, CL135, was isolated on the basis of its *cytR* phenotype according to the procedures of Hammer-Jespersen and Munch-Petersen (7) and Shimada et al. (25). After demonstrating that the *cytR* phenotype was due to lambda insertion within the gene (C. A. Liljestrand, Ph.D. thesis, Texas A&M University, College Station, 1983), specialized *met* transducing phage were isolated from lysates as described in the text.

prewarmed 0.01 M EDTA (pH 8.0) and held at 42°C for 10 min to enrich for deletion mutants (22). A plate lysate produced from the survivors was subjected to a second cycle of EDTA/heat inactivation under the same conditions. After preparing a plate lysate of the second-cycle EDTA survivors, deletion mutants were selected as follows: (i) λ p*met*100Δ1 (Table 1) was isolated by picking a nonhaloed plaque from a JJ131F lawn and constructing a JJ131F lysogen of that phage; (ii) λ p*met*100Δ2 (Table 1) was isolated by picking a haloed plaque from a comparable lawn, streaking for isolated colonies, and selecting for JJ131F lysogens of the transducing phage.

Construction of a JJ131F (λ p*met*100J5) lysogen. A λ p*met*100Δ1 lysogen of JJ119 was used to prepare a high-titer lysate. The lysate was plated on JJ131F cells according to the halo plaque assay procedure described herein. The goal was to select recombinant phage which replaced the deletion with the JJ119 *metB*$^+$ and *metJ185*(Am) alleles. Therefore, those rare plaques which exhibited haloes were picked into Luria broth medium. The cells which grew from the halo plaque picking were streaked for isolation and tested for lambda immunity. The resulting lysogens contain a prophage, λ p*met*100J5, which carries the *metB*$^+$ and the *metJ185*(Am) alleles (see Results).

Induction studies. JJ116 and JJ119 lysogens of λ p*met*100, λ p*met*100Δ1, and λ p*met*100Δ2 (Table 1) were used to analyze the expression of the phage-borne *metB* and *metJ* genes. Large-scale induction cultures of each of the lysogens were prepared according to described procedures (10), with the exception that, after 42°C induction, cultures were shifted to 39°C and samples were taken for enzyme assays at 30-min intervals. Cystathionine-*gamma*-synthetase and *beta*-cystathionase assays were performed on each sample taken during the induction period.

RESULTS

Transduction patterns. The plaque-forming transducing phage isolated during this study are listed in Table 1. Their *met* gene contents were first determined by their ability to transduce and complement a series of *E. coli met* mutants (Table 2). Each isolate successfully transduced JJ100 (*metB1*) to prototrophy, but failed to transduce JJ122 (*metF*). After several days, a few protorophic, presumed recombinants of RG776 (*metL*) were always observed in transduction experiments with λ p*met*100, -101, or -103, but not with λ p*met*102. As a positive control, comparable titers of λ d*met*128 (*metJ*$^+$*B*$^+$*L*$^+$*F*$^+$) (10, 14) were used to transduce JJ100, JJ122, and RG776 cells. In each case large numbers of transductants were observed (Table 2). This indicates that λ p*met*100, -101, and -103 do carry part of the *metL* gene, which codes for aspartokinase II/homoserine dehydrogenase II (27, 28).

TABLE 2. Transduction patterns of plaque-forming transducing phage

Phage	Relevant genotype	Transduction pattern[a]			
		No cell	JJ100 (*metB*)	RG776[b] (*metL*)	JJ122 (*metF*)
λ d*met*128[c]	*metJ*$^+$ *metB*$^+$ *metL*$^+$ *metF*$^+$	−	+	+	+
λ p*met*100	*metJ*$^+$ *metB*$^+$ *metL*[d]	−	+	∓	−
λ p*met*101, -103	*metJ*$^+$ *metB*$^+$ *metL*[d]	−	+	∓	−
λ p*met*102	*metJ*$^+$ *metB*$^+$	−	+	−	−

[a] Transductions were performed as described in the text. +, Heavy bacterial growth; −, no bacterial growth; ∓, sparse bacterial growth, presumably due to recombination between prophage and bacterial gene sequences.

[b] RG776 was supplemented with 0.2 mM L-threonine to compensate for the *metJ*$^+$ phenotype of transductants.

[c] λ d*met*128 (14) is a positive transduction control.

[d] The *metL* designation denotes that only a portion of the *metL* sequence is present.

Mapping of restriction sites. The extent and position of the bacterial DNA substitutions within the transducing phage chromosomes were determined by analyzing each DNA source with one or more of the following restriction endonucleases: *Eco*RI, *Hpa*I, *Sst*II, and *Sma*I. Because all of these transducing phage are *gal* types (Fig. 1), the right crossover point of phage/bacterial DNA is the attachment site locus (Fig. 2) (4, 24). Phage λ p*met*100 and -101/103 contain the *Sst*II restriction sites at lambda positions 0.419 and 0.423 (4, 24), but have no *Eco*RI sites in their left arm, indicating that the viral *Eco*RI sites at 0.438 and 0.538 (4, 24) are missing. The left crossover points of λ p*met*100 and -101/103 lie within the 0.73-kbp DNA sequence between the existing *Sst*II site at 0.423 and the missing *Eco*RI site at 0.438 (see Fig. 2). Phage λ p*met*102 contains the phage *Eco*RI site located at 0.438 λ units (Fig. 2), but is missing the *Hpa*I site at 0.452 (4, 24) (data not shown). The left crossover point of bacterial and phage DNA in λ p*met*102 lies within the 0.67-kbp DNA sequence between the described restriction sites.

Five *Sma*I restriction sites (three viral and two bacterial) are present in each of the plaque-forming *met* transducing phage chromosomes (Fig. 2) (4, 24). The *Sma*I sites create three unique restriction fragments (1, 2, and 3) which subdivide the bacterial DNA region (Fig. 2, Table 3). Fragment 1, which contains the *metJ* and *metB* genes, extends from the *Sma*I cut at lambda position 0.400 (4, 24) to the bacterial *Sma*I site approximately 1.1 kbp beyond *metJ*. Due to differences in bacterial and phage DNA content, the molecular weight of fragment 1 varies for each transducing phage. Fragments 2 (2.9 kbp) and 3 (4.4 kbp) are held in common by all of the transducing phage (Fig. 2, Table 3). Fragment 1 of λ p*met*102 contains approximately 2.0 kbp of *met* DNA which define only the *metB* and *metJ* genes and their control elements. Fragment 1 of both λ p*met*101 and λ p*met*103, which appear to be identical isolates, contains approximately 2.8 kbp of DNA from this region, and fragment 1 of λ p*met*100 contains 3.0 kbp of *met* DNA. The additional *met* DNA present in the λ p*met*100 and λ p*met*101/103 chromosomes defines a portion of the *metL* gene.

The *Sma*I restriction sites were also mapped in the previously isolated, defective *met* transducing phage λ d*met*128 and -117 (10, 14). The bacterial DNA carried by these phage chromosomes contains the intact *metJBLF* gene cluster inserted in the opposite orientation from the bacterial DNA carried by the plaque-forming *met* transducing phage described in this study (Fig. 1 and 2) (10, 14). Digestion of λ d*met*128 DNA with *Sma*I produces fragment 2 and a high-molecular-weight restriction fragment containing DNA extending from the lambda *Sma*I site at 0.653 λ units to the bacterial *Sma*I site just beyond *metJ* (Fig. 2). Phage λ d*met*117, which carries the intact *metJBLF* gene cluster but none of the *cytR* gene and very little bacterial DNA beyond the *metJ* gene (14, 28), does not contain any *Sma*I sites in the left arm of its chromosome (Fig. 2). This result confirms that the described bacterial *Sma*I site which lies at the juncture of fragments 1 and 2 is not in *metJ*.

EDTA/heat inactivation. The plaque-forming transducing phage and λ *c*I857 *S*am7 were exposed to 10 mM EDTA (pH

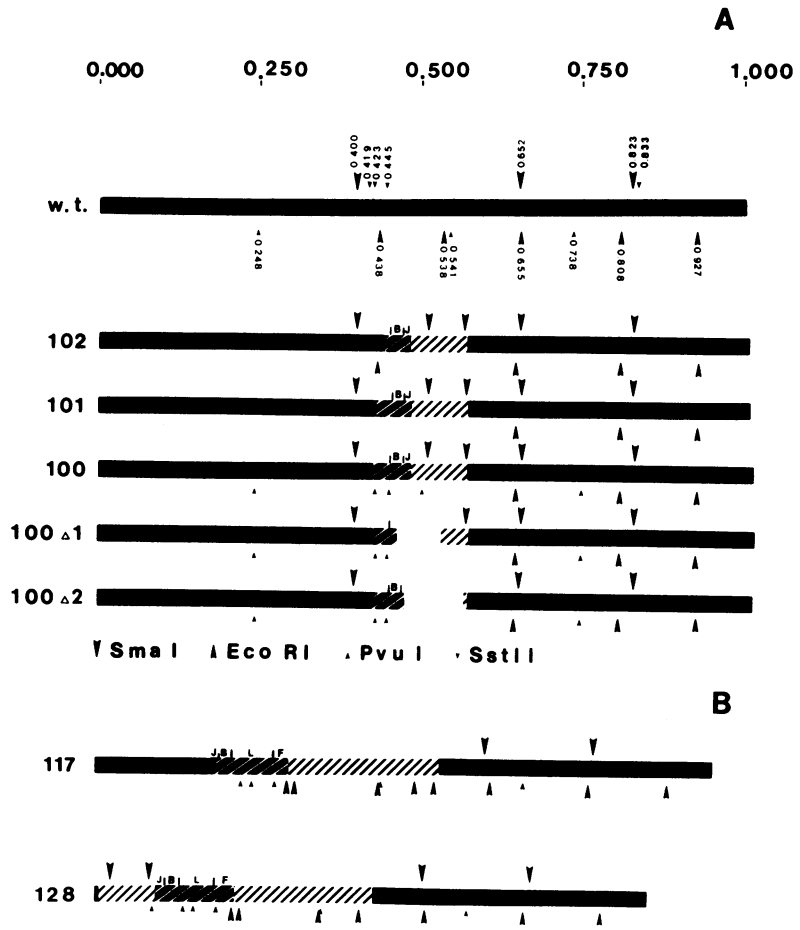


FIG. 2. Summary maps of the bacterial DNA content and the *Sma*I, *Eco*RI, and *Pvu*I restriction endonuclease sites of the specialized plaque-forming *met* transducing phage λ p*met*100Δ2, -100Δ1, -100, -101, and -102 (A) and of the previously isolated (10) defective transducing phage λ d*met*117 and -128 (B). Restriction sites are aligned relative to the chromosome of phage λ *c*I857 *S*am7 (wild type) (4, 24), and all positions are represented as decimal fractional lengths of phage λ. The dark bars represent bacteriophage DNA, and the hatched areas denote bacterial DNA. Light hatching on a dark background represents bacterial DNA which has been identified as a component of the *metJBLF* gene cluster (6, 10, 14, 28). The non-*met* bacterial DNA is represented by the dark hatching on a light background. The letter symbols indicate the general locations of the following genes: J, *metJ*; B, *metB*; L, *metL*; F, *metF*. The positions and proportions of each gene unit are based upon information presented in this paper and in references 6, 14, and 28 and unpublished studies of the *metF* gene by this laboratory.

8.1) at 42°C. The amount of phage inactivation that occurs during EDTA/heat shock is directly proportional to the molecular weight of the phage chromosome (22). From the results, it can be concluded that λ p*met*100 contains the largest chromosome, which is verified by its calculated molecular weight (Table 3). Because of this property, λ p*met*100 was chosen for deletion mutant selection. Before this study, additional characterization of λ p*met*100 was accomplished.

***met* gene expression and evidence that *metJ185* is an *amber* mutation.** Lysogens of λ p*met*100 and λ p*met*100J5 were constructed in JJ131 and JJ131F (Table 1), and cell cultures were grown on Luria broth, a medium which promotes repression of *met* regulon expression by *metJ*$^+$ strains (10, 26). Extracts of the JJ131(0) lysogen (cell/phage: *metJ184 metB1*/*metJ*$^+$ *metB*$^+$) contained enzyme activities comparable to the repressed values characteristic of extracts of the JJ116 (wild-type) strain (Table 4). Extracts of the JJ131(0J5) lysogen (cell/phage: *metJ184 metB1*/*metJ185 metB*$^+$) exhibited the expected derepressed levels of enzyme activity (Table 4). It was discovered that a λ p*met*100J5 lysogen of JJ131F, JJ131F(0J5) (cell/phage: *metJ184 metB1 supF*/*metJ185 metB*$^+$), possessed a *metJ*$^+$ phenotype, as indicated by repression of cystathionine-*gamma*-synthetase and *beta*-cystathionase specific activities. Since the JJ131F phenotype is *metJ* (Table 4), the *metJ185* defect must be due to an *amber* mutation. It can also be assumed that the *metB* alleles present in λ p*met*100 and λ p*met*100J5 are responsive to normal *met* regulon controls, including the *trans* action of the *metJ* gene product (10, 26).

Isolation and characterization of deletion mutants. The deletion mutants λ p*met*100Δ1 and λ p*met*100Δ2 were isolated as described in Materials and Methods. To confirm that these isolates were deletion mutants, their EDTA/heat inactivation profiles were determined at 42°C. Both λ p*met*100Δ1 and λ p*met*100Δ2 show less inactivation than λ p*met*100 (4.1 and 16.0% survivors versus 0.3% survivors, respectively), indirectly indicating that they have less chromosomal DNA.

When plated on JJ131F cells (halo plaque assay), λ p*met*100Δ1 plaques do not exhibit haloes, suggesting the absence of a functional phage-borne *metB* allele. When plated on JJ131F cells, λ p*met*100Δ2 plaques do produce

TABLE 3. Lengths of plaque-forming transducing phage restriction fragments[a]

Phage	EcoRI		SmaI		PvuI		Mol wt
	Fragment no.	Length	Fragment no.	Length	Fragment no.	Length	
λ pmet100	1	0.656	1	0.112	1	0.192	1.015
			2	0.059	2	0.018	
			3	0.088	3	0.051	
					4	0.246	
λ pmet101/103	1	0.654	1	0.110	Not done		1.013
			2	0.059			
			3	0.088			
λ pmet102	1	0.211	1	0.108	Not done		1.011
			2	0.059			
			3	0.088			
λ pmet100Δ1	1	0.583	1	0.098	1	0.192	0.942
			2	0.088[b]	2	0.018	
					3	0.224	
λ pmet100Δ2	1	0.559	1	0.163	1	0.192	0.918
					2	0.018	
					3	0.200	
λ dmet128[c]	1	0.234	1	0.041	1	0.106	0.850[d]
	2	0.006	2	0.059	2	0.051	
	3	0.114	3	0.403	3	0.018	
	4	0.056			4	0.037	
	5	0.102			5	0.136	
					6	0.231	

[a] Only the lengths of restriction fragments which contain bacterial DNA are listed. Fragments are numbered in order, from left to right, as they appear within the restriction maps of the vegetative phage chromosomes (see Fig. 2). The lengths of restriction fragments are presented as decimal fractions of the molecular weight of λ (4).

[b] This fragment is identical to fragment 3 of λ pmet100, -100/103, and -102.

[c] The EcoRI restriction fragment lengths of λ dmet128 are those reported in Krueger et al. (14).

[d] The molecular weight is based upon the sum of the SmaI restriction fragment lengths and compares favorably with the molecular weight of 0.858 λ units which was reported in reference 14.

haloes, indicating that this phage contains a functional *metB* gene. This conclusion was confirmed by measuring the cystathionine-*gamma*-synthetase activity present in crude extracts of Luria broth cultures prepared from λ pmet100Δ1 or λ pmet100Δ2 lysogens of strains JJ100F and JJ131F (Table 1) (data not shown).

Phage λ pmet100, λ pmet100Δ1 or λ pmet100Δ2 lysogens of JJ116 and JJ119 were constructed (Table 1) and used to conduct prophage induction studies of *metB* gene expression (see Materials and Methods). The results of these studies are plotted in Fig. 3 as cystathionine-*gamma*-synthetase specific activity versus time postinduction. Extracts of λ pmet100 lysogens of JJ116[JJ116(0)] and JJ119[JJ119(0)] show no significant time-dependent increase in enzyme specific activity. However, there is a slightly higher level of specific activity in extracts of JJ119(0) which is presumably due to the presence of only one $metJ^+$ allele in the strain. These results suggest that phage promoters contribute only minimal levels of *metB* gene expression during induction in the presence of a functional *metJ* gene product. Extracts of the JJ116 (0Δ1) were found to have intermediate levels of cystathionine-*gamma*-synthetase activity as compared with samples prepared from JJ116(0) and JJ119(0) lysogens. This result is probably due to the presence in the JJ116(0Δ1) strain of only one $metB^+$ and $metJ^+$ allele. When no $metJ^+$ allele is present, as in strain JJ119(0Δ1), the cystathionine-*gamma*-synthetase specific activity in extracts exhibits a net fourfold increase over the level of activity associated with samples prepared from the JJ116(0Δ1) strain, presumably due to the effect(s) of the induction-associated temperature shift. The differences in the levels of induced specific activity observed in extracts of strains JJ119(0) and JJ116(0Δ2) are assumed to be due to the properties of the bacterial *metJ* alleles, to the deletion of the prophage-borne *metJ* gene (which also moves the *metB* gene copy closer to the phage promoters p_{int} and p_L), and to temperature shift effects. When the λ pmet100Δ2 prophage is present in strain JJ119[JJ119(0Δ2)] or JJ116[JJ116(0Δ2)], the net result of induction is a 10- to 15-fold increase in JJ119(0Δ2)-associated enzyme specific activity as compared with the specific activity produced by the corresponding JJ116(0Δ2) extract [a result which is primarily due to the absence of a functional *metJ* gene product in the JJ119(0Δ2) strain]. The initial *beta*-cystathionase specific activities of these strains are comparable to their *metJ* gene content and do not increase during the induction period (data not shown). These observations confirm that the *metB* allele of the λ pmet100Δ2 chromosome retains regulatory regions which are sensitive to the *metJ* gene product.

Nuclease analysis of deletion mutants. To describe the deletions present in λ pmet100Δ1 and λ pmet100Δ2, SmaI and PvuI restriction digests of the phage DNAs were analyzed. In λ pmet100Δ1, SmaI fragments 1 and 2 of the λ pmet100 restriction pattern have been replaced by a 4.8-kbp fragment. This result indicates the loss of one bacterial SmaI site and the deletion of 3.6 kbp of bacterial DNA (Fig. 2, Table 3). Phage λ pmet100Δ2 has lost both bacterial SmaI sites due to the deletion of 4.75 kbp of DNA. This deletion eliminates SmaI fragments 1, 2, and 3 and creates a new, 8.05-kbp restriction fragment (Fig. 2, Table 3). The PvuI restriction map of λ dmet117/102 was recently reported by Zakin et al. (28). Phage λ pmet100 was found to have two of the three PvuI sites present in the bacterial DNA of λ dmet117, as well as a new PvuI site which is present in the bacterial DNA between *metJ* and *cytR*, but which λ dmet117/102 does not carry (Fig. 2). This PvuI site is also present in the λ dmet128 chromosome (Fig. 2). Zakin et al. (28) reported a PvuI site at a similar location in the chromosome of λ h80 dmetA4, but did not resolve its phage or bacterial origin. Due to their deletions, neither λ pmet100Δ1 nor λ pmet100Δ has this PvuI site. However, each deletion mutant retains the two closely spaced bacterial PvuI restriction sites of λ pmet100 (Fig. 2). Therefore, the λ pmet100Δ1-associated deletion begins within, or just beyond, the *metB* gene and extends through the *metJ* gene and beyond the SmaI site just to the right of *metJ* (see Fig. 2). The deletion in λ pmet100Δ2 must be located between the 0.473 and 0.534 positions on the λ pmet100 chromosome because the *metB* gene and its regulatory units are still present. Collectively, these data indicate that the λ pmet100Δ2-associated deletion extends from just before the phage/bacterial hybrid attachment site to within at least 440 bp of the *metB* gene (see Fig. 2).

DISCUSSION

The regulatory properties of λ pmet100Δ2 prove that the *metB* gene can be expressed independently of other genetic elements of the *met* regulon and indicate that the *metJ* gene could not be a promoter-proximal element of a *metJB*

TABLE 4. Enzyme activities in extracts of JJ131 and JJ131F lysogens[a] of λ p*met*100 and λ p*met*100J5

Strain	Relevant genotype (cell/phage)	Sp act (nmol/min per mg of protein)	
		Cystathionine-*gamma*-synthetase	*Beta*-cystathionase
JJ116	*metJ*$^+$ *metB*$^+$	0.3	1.3
JJ119	*metJ185*(Am) *metB*$^+$	11.3	25.3
JJ131	*metJ184 metB1*	0.2	7.1
JJ131F	*metJ184 metB1 supF*	0.3	7.0
JJ131(0)	*metJ184 metB1*/*metJ*$^+$ *metB*$^+$	0.2	1.3
JJ131(0J5)	*metJ184 metB1*/*metJ185*(Am) *metB*$^+$	7.4	5.6
JJ131F(0)	*metJ184 metB1 supF*/*metJ*$^+$ *metB*$^+$	0.2	1.5
JJ131F(0J5)	*metJ184 metB1 supF*/*metJ185*(Am) *metB*$^+$	0.3	1.5

[a] All assays were performed with cell extracts prepared from cultures grown as described in the text and in reference 10. The medium was Luria broth, and cultures were grown at 32°C to an optical density of 1.0 (550 nm). Normally, these growth conditions lead to repression of *met* regulon gene activity (10).

transcription unit. The discovery that the *metJ185* defect is due to an *amber* mutation also argues against this possibility, since no polar effects (reviewed in reference 1) on *metB* expression are observed in strains which contain this mutation (Table 4). Isolation of the *metJ185*(Am) allele also confirms earlier observations of Minson and Smith (20) which were made with a *metJ*(Am) mutant of *Salmonella typhimurium*. The report of Greene et al. (6) indicates that the *metB* and *metL* genes form a transcription unit. Zakin et al. (27, 28) demonstrated that the polarity of *metL* expression is clockwise on the linkage map of *E. coli* (2) and that no promoter is closely associated with the *metL* sequence. Therefore, the *metJ* gene must belong to a transcription unit which is independent of the *metBL* or *metF* unit. The observations of Mulligan et al. (21) are consistent with this conclusion.

The deletion mutants λ p*met*100Δ1 and λ p*met*100Δ2 confirm the gene order of *metJBLF* when moving in a clockwise direction on the standard recombination map of *E. coli* K-12 (2). The deletion in λ p*met*100Δ1 removes the entire *metJ* sequence and is the first reported *metJ* mutation of this type. The λ p*met*100Δ2 deletion removes only the left component of the *metJ* region (clockwise orientation on the *E. coli* K-12 linkage map [2]) (Fig. 2). These deletion mutations and the *metJ185*(Am) allele associate the *metJ* phenotype (20, 26) with an absence, or failure, of function of the *metJ* gene product. This conclusion is supported by the fact that suppression of the *metJ185*(Am) mutation by the

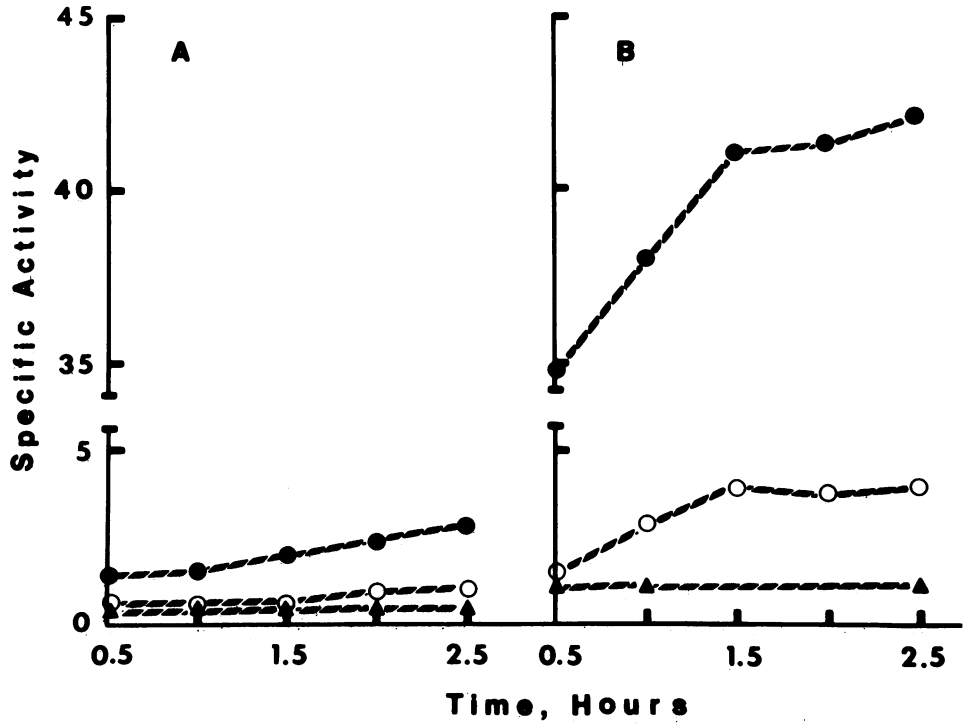


FIG. 3. Expression of the *metB* gene (cystathionine-*gamma*-synthetase) during prophage induction. Specific activity is expressed in nanomoles per minute per milligram of protein, and time represents the number of hours postinduction (see text for the induction procedure). (A) Results of induction of a series of JJ116 (*metJ*$^+$ *metB*$^+$ lysogens; (B) a similar series of inductions of JJ119 [*metJ185*(Am) *metB*$^+$] lysogens. Symbols: ▲, JJ116(0)/JJ119(0) = λ p*met*100 lysogens; ○, JJ116(0Δ1)/JJ119(0Δ1) = λ p*met*100Δ1 lysogens; ●, JJ116(0Δ2)/JJ119(0Δ2) = λ p*met*100Δ2 lysogens.

supD or *supE* allele results in the synthesis of partially active, temperature-sensitive gene products (unpublished data, this laboratory).

Based upon the pattern of *metB* gene expression during induction of lysogens of λ p*met*100, -100Δ1, and -100Δ2, the chromosomal *metJ*$^+$ allele retains considerable control over the expression of the amplified *metB* copies (Fig. 3). If the prophage contains a *metJ*$^+$ allele, there appears to be a balance between the expression of *metJ* and the number of *metB*-associated regulatory regions. Therefore, only in the absence of a functional *metJ* allele is there significant expression of the amplified *metB* gene copies and accumulation of cystathionine-*gamma*-synthetase activity. These results indicate that the *metJ* gene product probably functions immediately upon its synthesis.

Previously, the physical structure of the *metJBLF* gene cluster was studied through the use of two independently constructed series of specialized lambda transducing phage (6, 10, 14, 23). The plaque-forming *met* transducing phage described in this report represent an independent, third series of phage vectors containing portions of the *metJBLF* gene cluster. Analysis of these isolates confirmed the gene order and demonstrated the independent transcriptional organization of the *metJ* and *metB* genes. The restriction maps of these transducing phage establish the bacterial DNA sequence identity of the *Pvu*I site described by Zakin et al. (28). The *Sma*I restriction sites discovered within the bacterial DNA sequences of the plaque-forming transducing phage have been used to align the restriction maps of plaque-forming isolates, of deletion derivatives, and of previously isolated defective transducing phage (Fig. 2). Collectively, these results provide a basis for more detailed studies of these *met* regulon transcription units.

ACKNOWLEDGMENTS

We thank Donna Canter and Olin K. Fearing for technical assistance.

This work was supported by Public Health Service research grant GM 28214 from the National Institutes of Health and by National Institutes of Health Biomedical Research Support Grant 55478 from Texas A&M University to J.R.J.

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