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NOTES

Insertion Mutagenesis of the *metJBLF* Gene Cluster of *Escherichia coli* K-12: Evidence for an *metBL* Operon

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The effects of Mu or transposon *5* insertions on the expression of genes of the *metJBLF* cluster show that *metB* and *metL* form an operon, transcribed from *metB* to *metL*, and that *metF* and *metJ* are independently transcribed.

Most of the *met* regulon genes are scattered on the *Escherichia coli* chromosome (2). However, *metB*, *metF*, and *metL* are clustered in a segment of DNA about 5 kilobases long (8, 20). Several workers have shown that *metB* and *metF* are in different transcriptional units (5, 12–15, 17), but *metL* has not been examined. Since *metL* and *thrA* each code for an aspartokinase-homoserine dehydrogenase (16), a strain with a mutation in *thrA* is required before *metL* expression can be evaluated. We have examined the effect of insertion mutations on the expression of genes in the cluster (R. C. Greene and A. A. Smith, Fed. Proc. **41**:752, 1982) in strain Gif881L (*metJ1001 thrA1000*) (16).

Strain Gif881L was infected with Mu *c*ts61 (10), and mutants that required methionine or threonine or both were isolated by ampicillin selection. The mutants were classified by their growth requirements, and those of interest were tested for complementation by λ d*met*117, which carries the entire *metJBLF* cluster (11). Of several hundred mutants examined, those resulting from Mu insertion into the *metBLF* cluster included *metF* mutants (growth only on methionine), *metL* mutants (growth on homoserine), and *metBL* mutants (both cystathionine and homoserine required), but no single *metB* mutants. Further study of *metF* and *metL* strains was limited to those in which the Mu insertion was associated with the auxotrophic mutation, as judged by the simultaneous loss of the prophage and the nutritional requirement after transduction with P1.L4 (4). In contrast, each mutation that gave a MetBL phenotype was transferred to a *metF*::Mu derivative of strain Gif881L (RG781) by P1 transduction. Transductants were selected on medium supplemented with cystathionine and threonine. All the auxotrophic transductants were shown to be single lysogens with Mu inserted in the *met* gene cluster. Auxotrophs from one of the transductions grew on homoserine alone, indicating that the donor strain carried mutations in both *metB* and *metL*, but those from all the other transductions still required both supplements. Since these and other transductions failed to separate the MetB and MetL phenotypes, it appears that the double growth requirement does result from a single mutational event.

Extracts of strain Gif881L and several independent Mu insertion mutants were assayed for the products of the *metB*, *metF*, and *metL* genes (Table 1). The *metF* strain (RG781) had no 5,10-methylenetetrahydrofolate reductase activity, but the other two enzymes had activities comparable to those in the parent strain, Gif881L. In the strains with Mu inserted in *metL*, the *metB* and *metF* enzymes had high activities, but the level of homoserine dehydrogenase was greatly reduced. Finally, the presumptive *metB* insertion lysogens had no cystathionine synthetase activity, but were otherwise similar to the *metL* mutants.

During the work, we observed that strain Gif881L excreted methionine precursors into the medium, which cross-fed the methionine auxotrophs and reduced the yield of mutants obtained by ampicillin selection. Since this phenomenon may have selectively eliminated single *metB* mutants, we thought it was worthwhile to examine Mu insertions in *metB* isolated from a different strain. The *metB*::Mu insertions of two strains (MH4166 and MH4170), obtained from Martha Howe, University of Wisconsin, and the *metB1* mutation were transferred into strain RG781 as described above to yield strains in which *metL* expression could be measured. All the transductants received functional alleles of the closely linked *metJ* gene. Elevated levels of 5,10-methylenetetrahydrofolate reductase in strains grown on D-methionine sulfoxide showed derepression of the regulon (Table 2). The *metB1* strain (RG733) was also derepressed for *metL*, and the two Mu mutants had similarly low homoserine dehydrogenase activity in extracts of both repressed and derepressed cells. Thus, although strains MH4166 and MH4170 were isolated as single *metB* mutants, their Mu insertions interfered with *metL* expression. In contrast, the insertions had no effect on *metF* or *metJ*.

To obtain polar mutations that could be examined at the molecular level, we constructed several transposon *5* (Tn*5*) insertion derivatives of the *metJBLF* transducing phage λ d*met*102 (11). The structure of each modified phage was determined by restriction fragment mapping (Fig. 1) (1, 12, 20). Three of the independently isolated insertions were in the *metB* coding sequence, and one was in *metF*. Except for the Tn*5* insertions, there were no obvious abnormalities, and the segment of DNA containing *metL* was present. When λ d*met*102 *metF*::Tn*5* was cross-streaked against a set of *met* mutants, it complemented the *metB* and *metL* strains but not the *metF* strains. All the λ d*met*102 *metB*::Tn*5* phage strongly complemented the *metF* strains, did not complement the *metB* strains, and weakly complemented the *metL* strains. Even when the λ d*met*102 *metB*::Tn*5* phage were cross-

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TABLE 1. Gene expression in Mu insertion mutants

Strain[a]	Site of Mu insertion	Activity (nmol/min per mg of protein)[b]		
		Cystathionine synthetase (*metB*)	Homoserine dehydrogenase (*metL*)	*N*-5,10-Methylene tetrahydrofolate reductase (*metF*)
Gif881L		54	115	2.8
RG704	*metB*	0.02	3.6	5.0
RG706	*metB*	−0.07	4.0	6.1
RG742	*metL*	28	2.7	5.1
RG750	*metL*	28	2.8	4.7
RG781	*metF*	45	101	0.00

[a] The strains in which Mu is inserted in the same gene were obtained by separate Mu infections.

[b] Procedures for growing cells, assaying enzymes, and determining protein content have been described previously (6, 7, 9, 11, 18).

TABLE 2. Gene expression in *metB metJ*$^+$ transductants

Strain and growth conditions[a]	*metB* mutation (strain)	Activity (nmol/min per mg of protein)		
		Cystathionine synthetase (*metB*)	Homoserine dehydrogenase (*metL*)	*N*-5,10-Methylene tetrahydrofolate reductase (*metF*)
Repressing				
RG731	*metB*::Mu (MH4166)	0.00	4.4	0.04
RG732	*metB*::Mu (MH4170)	0.00	4.5	0.06
RG733	*metB1*	0.00	6.3	0.05
Derepressing				
RG731	*metB*::Mu (MH4166)	−0.16	3.8	7.1
RG732	*metB*::Mu (MH4170)	−0.03	3.8	8.3
RG733	*metB1*	0.07	66.4	6.9

[a] Cultures were grown on minimal dextrose-thiamine medium supplemented with 0.5 mM L-threonine and either 0.5 mM L-methionine for repressing conditions or 1 mM D-methionine-DL-sulfoxide for derepressing conditions.

streaked against *recA* derivatives of *metL* strains, occasional prototrophic colonies grew. We therefore constructed λ d*met*102 *metB*::Tn*5* lysogens of *metB* and *metL* strains and measured enzyme activities in extracts of cells grown under repressing and derepressing conditions. The Tn*5* insertions reduced the expression of the phage-borne *metL* genes without affecting the expression of the chromosomal *metL* gene of strain RG733 (Table 3).

Although *metL* expression was greatly reduced in *metB* insertion mutants, there were indications of residual low-level transcription. When about 10^8 cells of a *metB* insertion mutant (either Mu or Tn*5*) were spread on a plate containing methionine but not homoserine, a background haze of growth and hundreds of revertants appeared after a few days of incubation. This behavior depended on the presence of a functional *metL* gene. The low-level transcription could arise from a secondary promoter or possibly from the insertion element (3), but regardless of its origin it provides evidence that *metL* can be translated when *metB* is not being expressed. The rare colony that grew when λ d*met*102 *metB*::Tn*5* was cross-streaked against a *metL recA* strain could be a polylysogen in which several copies of a poorly expressed *metL* gene provide enough enzyme for growth.

The results suggest that *metB* and *metL* form an operon transcribed from *metB* to *metL*. This is consistent with the sequence information (5, 19), which shows that the genes are

TABLE 3. Gene expression in λ d*met*::Tn*5* lysogens

Bacterial strain	Cellular *met* mutations	Growth conditions[a]	Activity (nmol/min per mg of protein)		
			Cystathionine synthetase (*metB*)	Homoserine dehydrogenase (*metL*)	*N*-5,10-Methylene tetrahydrofolate reductase (*metF*)
Nonlysogens					
RG733	*metB*	R	0.0	2.2	0.2
RG775 *recA56*	*metL metJ*	R	36	0.0	4.9
RG733	*metB*	D	−0.1	83	5.3
RG775 *recA56*	*metL metJ*	D	41	1.0	7.3
Lysogens					
λ d*met*102 *metB*::Tn*5*-2					
RG733	*metB*	R	0.1	6.5	0.2
RG775 *recA56*	*metL metJ*	R	1.1	1.7	0.7
RG733	*metB*	D	0.1	123	15.4
RG775 *recA56*	*metL metJ*	D	22	3.6	12.9
λ d*met*102 *metB*::Tn*5*-6					
RG733	*metB*	R	0.2	4.6	0.2
RG775 *recA56*	*metL metJ*	R	0.5	4.2	0.2
RG733	*metB*	D	−0.1	108	14.4
RG775 *recA56*	*metL metJ*	D	20	6.1	16.6
λ d*met*102 *metB*::Tn*5*-18					
RG733	*metB*	R	0.0	7.5	0.1
RG775 *recA56*	*metL metJ*	R	0.9	2.3	0.4
RG733	*metB*	D	0.1	119	12.4
RG775 *recA56*	*metL metJ*	D	15	2.2	14.2

[a] Cells were grown on minimal dextrose medium supplemented with thiamine, 0.5 mM L-threonine, and either 0.5 mM L-methionine for repressing conditions (R) or 1 mM D-methionine-DL-sulfoxide plus 5μM D-methionine for derepressing conditions (D).

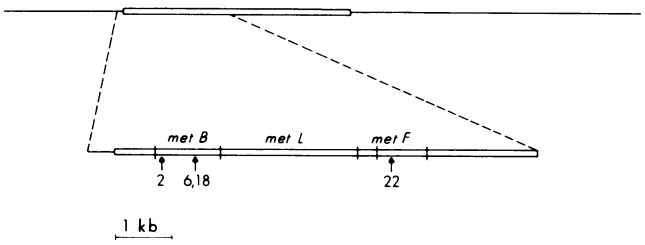

FIG. 1. Locations of Tn*5* insertions in λ d*met*102. Numbers indicate the particular Tn*5* insertion mutation. The sites of insertion were mapped by measuring the fragment lengths obtained after digestion with various combinations of *Eco*RI, *Hin*dIII, *Pvu*I, *Pvu*II, and *Xho*I. The positions of the *metB*, *metL*, and *metF* genes are taken from their published DNA sequences (5, 15, 19). ————, λ DNA; ▭, bacterial DNA; ↑, site of Tn*5* insertion.

very close together and are read in the same direction. Although the *metJ* and *metF* genes are closely linked on either side of the *metBL* operon, this expression was not affected by the insertion mutations, and they appear to be in separate transcriptional units.

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