Cloning of the Methionine Regulatory Gene, \textit{metJ}, of \textit{Escherichia coli} K12 and Identification of Its Product*

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Both wild-type and mutant forms of the methionine regulatory gene, \textit{metJ}, of \textit{Escherichia coli} K12 have been cloned in derivatives of pBR322. In cells carrying plasmids with a functional copy of \textit{metJ}, the methionine regulon appears to be repressed even under conditions of methionine limitation. Maxicell labeling experiments show that the plasmids code for a small peptide (12 kilodaltons) only when they carry a functional copy of \textit{metJ}. The lesions in five independently isolated \textit{metJ} mutants are located in, or slightly upstream from, a coding sequence proposed to be \textit{metJ} by Saint-Girons, I., Duchange, N., Cohen, G. N., and Zakin, M. M. ((1984) \textit{J. Biol. Chem.} 259, 14282–14285).

The genes for methionine biosynthetic enzymes are scattered on the \textit{Escherichia coli} chromosome (1). Only two of them, \textit{metB} and \textit{metL}, are known to be in an operon (2–4), and the others are independently transcribed. The expression of all of them is controlled by a major regulatory system defined by mutations in \textit{metJ} and \textit{metK} (5). On the map of the \textit{E. coli} chromosome, the \textit{metJ} gene is very close to \textit{metB} (1). During our work on cloning of the \textit{metBLF} gene cluster, we also cloned \textit{metJ} (6–8). In this paper, we describe the construction of several plasmids with wild-type and mutant alleles of \textit{metJ}, and identify the peptide product of the cloned genes. We further show that high-copy-number plasmids with functional \textit{metJ} alleles can cause a methionine-growth requirement, apparently because of overproduction of the gene product and repression of the regulon. We have used this property to isolate insertion mutations of the plasmid-borne \textit{metJ} genes.

**MATERIALS AND METHODS**

**RESULTS**

**Structures of the Plasmids**—We had previously cloned a segment of a \textit{met}-transducing phage (\textit{Xdmetl02}) into the relatively low-copy-number plasmid pVH2124 (8). One of these plasmids (\textit{pRCG112}) was shown to carry functional copies of \textit{metB}, \textit{metF}, and \textit{metL}. In addition, it was presumed to carry \textit{metJ}, because of the gene content of the parent transducing phage (6, 7, 24). As described under "Materials and Methods," two overlapping segments of \textit{pRCG112} were subcloned into pBR322 to give \textit{pRCG131}, \textit{pRCG134}, and \textit{pRCG151}. The structures of the plasmids, determined by restriction-fragment mapping, are diagrammed in Fig. 1. Using the mapping data and information about the sequences of \textit{X} and the \textit{met} genes (25–27), we have located the exact positions of the ends of the segments of bacterial and viral DNA inserted into pBR322. The inserts of the \textit{pRCG130} series begin at the \textit{PstI} site at base number 5686 of \textit{X} and, after 2.69 kb of \textit{X} DNA and 2.08 kb of bacterial DNA, end at the \textit{Poul} site at position 198 of the \textit{metL} structural gene. The insert of \textit{pRCG151} begins at the \textit{HincII} site at base number 8201 of \textit{X}, proceeds through 0.27 kb of \textit{X} DNA and 4.56 kb of bacterial DNA, and ends at the \textit{HincII} site at base number 9 in the promoter of \textit{metF}.

We also constructed plasmids with mutant alleles of \textit{metJ}, which, except for the mutations, have the same structures as \textit{pRCG131} and \textit{pRCG134}. \textit{pRCG135} carries \textit{metJ184} and \textit{pRCG137} carries \textit{metJ185} (which has been shown to be an amber mutation (13)). Appropriate regions of these plasmids have been sequenced to determine the nucleotide changes responsible for the mutant phenotypes (28).

**Growth Behavior and Enzyme Activities of Transformants**—Transformants of strain TS214 (\textit{metB1, pok4ts214}) with any of the plasmids have the expected antibiotic resistances and will grow on methionine-free medium. Thus, as expected, they all have a functional copy of \textit{metB}. In addition, transducing phage constructed by insertion of the \textit{EcoRI} fragment of \textit{pRCG151} into \textit{Xgt2} (29) will complement both \textit{metB} (including strain \textit{JJ100}) and \textit{metL} mutants. However, when strain \textit{JJ100} is used as the recipient, reasonable numbers of antibiotic-resistant transformants are obtained from all of the plasmids, but only \textit{pRCG137} (\textit{metJ185}) gives a significant number of prototrophs. When large numbers of \textit{JJ100} cells transformed with the \textit{metJ*} plasmids are spotted on minimal plates without antibiotic, colonies slowly arise. Several of these colonies were examined and found to be \textit{metB*} recombinants that had lost the plasmid. Furthermore, transformants of strains \textit{JJ100} or \textit{MS100} (\textit{metB1, reBC}) with \textit{pRCG131}, \textit{pRCG134}, or \textit{pRCG151}, won't grow on cystathionine which will support the growth of the parent strains. Similarly, transformants of strain \textit{C600 recBC} with these plasmids require methionine for growth, even though this amino acid can be synthesized by the plasmid-free strain.
These observations suggest that the plasmids cause overproduction of the *metJ* gene product which shuts down expression of the *met* regulon. To test this hypothesis, we grew several transformants of strain MS100 on minimal medium supplemented with either 1 mM L-methionine (repressing conditions) or 1 mM D-methionine-DL-sulfoxide (derepressing conditions (30)). Sonicate extracts of the transformants were assayed for the products of the *metB*, *metC*, *metF*, and *metK* genes. As shown in Table 1, the pBR322 transformant of MS100 shows the expected behavior, with methionine limitation causing significant derepression of *metC*, *metF*, and *metK* (7-30-fold). In contrast, the transformants containing plasmids with functional *metJ* genes show little or no derepression of the *metC* or *metF* genes and small increases (2-4-fold) in the activity of the *metK* gene product. The transformants show somewhat larger increases in expression of the plasmid-borne *metB* gene, but that may partially result from amplification of the plasmid, since the *metJ*+ transformants grow very slowly on D-methionine sulfoxide.

**Isolation and Characterization of Insertion Mutations of metJ**—We used the growth behavior of the MS100 (*metB*, *recBC*) transformants to isolate mutations of the plasmid-borne *metJ* genes. We selected spontaneous mutants of pRGC131, pRGC134, and pRGC151 transformants which could synthesize methionine, but still contained plasmids, by preventing detection of the gene to be a small peptide. All five of the independently isolated mutations of each type of plasmid was purified and characterized, restriction fragment mapping showed that all three of the mutant plasmids had 'Tn1000'(31, 32) inserted near the counterclockwise ends of their bacterial DNA segments. As diagrammed in Fig. 2, each of the inserts was located in or slightly upstream from an open reading frame selected by Saint-Girons et al. (28) as the *metJ* coding sequence.

**Identification of the metJ Peptide**—The peptides synthesized under the direction of plasmid genes were labeled by the maxicell procedure (20) in transformants of strain SR58. Extracts of cells carrying plasmids with a functional *metJ* allele contain a low-molecular-weight radioactive peptide that has a molecular mass of 12 kDa on a sodium dodecyl sulfate slab gel, which is consistent with the predictions of Saint-Girons et al. (28), whose sequence data suggest that the *metJ* peptide has a molecular mass of 12 kDa.

**DISCUSSION**

The results presented here show the product of the *metJ* gene to be a small peptide. All five of the independently isolated mutations are located in, or slightly upstream from, the *metJ* coding sequence proposed by Saint-Girons et al. (28). The locations of these mutations and the apparent size of the peptide are consistent with their selection of the *metJ* reading frame. Detection of the *metJ* gene product was facilitated by use of a high-copy-number plasmid that causes its overproduction. Such overproduction, however, turns off the *met* regulon (even under conditions of methionine limitation), preventing detection of *metJ* structural genes carried on the same plasmid. This growth behavior may account for the failure to find a *metB* plasmid among the Clarke and Carbon collection (30), since the plasmid would be likely to carry both *metB* and *metJ*.

The original cloning of the *metJ* gene cluster was done in two ways (8). One used a relatively low-copy-number vector (pVH2124), while the other used pBR322, but screened transformants in a strain with a ts mutation in polA (TS214). In either case, transformants probably have too few copies of the *metJ* gene to turn off the regulon, allowing complementation by the plasmid-borne *metB* gene. We have used this property of strain TS214 to show that all of our plasmids had functional *metB* genes, even though most of them would not transform strain JJ100 to prototrophy.

The mechanisms involved in control of expression of the *met* regulon are unknown, but the growth behavior and enzyme activities of some of the plasmid-bearing cells described here, together with the observation that cells with an amber mutation of *metJ* are derepressed (19), suggest that the *metJ* peptide is a negative effector, perhaps a simple repressor.

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**REFERENCES**

metJ Gene Product of E. coli K12

COUPLING OF THE METHIONINE REGULATORY OPERON, metJ, OF E. COLI K12 AND IDENTIFICATION OF ITS PRODUCT

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BACTERIUM AND PHAGE. Strain J3320 (metM, recA, thr) has been described (6). A reovirus phage of J3320 (MCS20) was constructed by a modification of a published procedure (7). The bacterial strain was grown on Brain Heart Infusion Broth (Difco), 100 mg L-1 of 13C-labeled threonine (Cambridge Isotope Laboratories), 1.5 mg L-1 of 13C-lysine (Cambridge Isotope Laboratories), and 1.5 mg L-1 of 13C-tryptophan (Cambridge Isotope Laboratories). The bacteria were harvested after 18 h of incubation at 37°C, washed once with saline, and resuspended in M9 medium (8) containing 2.5% methionine-15N (Cambridge Isotope Laboratories) and 1.5 mg L-1 of 13C-lysine (Cambridge Isotope Laboratories). The culture was then incubated for 3 h at 37°C before harvesting. The bacteria were harvested by centrifugation, washed once with saline, and resuspended in M9 medium containing 2.5% methionine-15N (Cambridge Isotope Laboratories) and 1.5 mg L-1 of 13C-lysine (Cambridge Isotope Laboratories). The culture was then incubated for 3 h at 37°C before harvesting.

Chemicals. Restriction enzymes were obtained from Bethesda Research Laboratories (Bethesda, MD) and from New England Biolabs (Beverly, MA). Recombinant enzymes were obtained from New England Biolabs (Beverly, MA) and from New England Biolabs (Beverly, MA).

General Methods. Growth of cells and assay of the enzymes coded by the metB, metC, metE, and metF genes has been described previously (9, 10, 11, 12). Except for wild, all restriction enzymes were done in 0.5× buffer containing Tris-HCl pH 7.5, NaCl, MgCl2, Na2EDTA, and RNase inhibitor, and the results were assayed by agarose gel electrophoresis. The results were analyzed by gel densitometry. The nucleotide sequence of the metJ gene and the DNA fragments were determined as described by Maniatis et al. (11) except that the cDNA was hybridized to DNA probes. The DNA fragments were hybridized to DNA probes that were labeled with 32P by the random priming method (13) and then hybridized to DNA probes that were labeled with 32P by the random priming method (13). The DNA fragments were hybridized to DNA probes that were labeled with 32P by the random priming method (13). The DNA fragments were hybridized to DNA probes that were labeled with 32P by the random priming method (13).

Purification of metJ. Purification of metJ was performed by the method of Elson et al. (14) using 10× and 2× lysis buffer. The metJ protein was then purified to homogeneity by SDS-PAGE on a 12% acrylamide gel. The metJ protein was then purified to homogeneity by SDS-PAGE on a 12% acrylamide gel. The metJ protein was then purified to homogeneity by SDS-PAGE on a 12% acrylamide gel. The metJ protein was then purified to homogeneity by SDS-PAGE on a 12% acrylamide gel.