The *Escherichia coli* K-12 *metJ193* Allele Contains a Point Mutation Which Alters the Hydrophobic Pocket Responsible for In Vitro Binding of S-Adenosylmethionine: Effects on Cell Growth and Induction of *met* Regulon Expression

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The *metJ193* allele encodes one of two identified temperature-sensitive *Escherichia coli* K-12 *met* repressors. The nucleotide sequence of the *metJ193* allele was determined. The point mutation was a T to A transversion at base 170 of the *metJ193* open reading frame and resulted in the substitution of leucine by glutamine at the 56th amino acid residue of the MetJ193 protein. The mutational lesion altered the hydrophobic pocket responsible for in vitro binding of the corepressor S-adenosylmethionine by wild-type MetJ. MetJ193 protein formed at the permissive temperature (28°C) allowed slow derepression of *met* regulon expression when cultures were shifted to the nonpermissive temperature (34°C). When 28°C cultures of strains bearing two *metJ193* alleles were transferred from methionine-containing medium to minimal medium, derepression of *met* regulon expression did not occur quickly enough to avoid a lag in growth due to the methionine deprivation. The inability of the MetJ193 protein to easily accomplish transition between apo- and active-repressor conformations was also demonstrated by using a maxi-cell system to study expression of a plasmid-borne copy of the *E. coli* *metF* transcription unit. These results confirm the importance of the leucine 56 residue for the structure and function in vivo of the wild-type MetJ protein.

In *Escherichia coli* and *Salmonella typhimurium*, methionine biosynthesis utilizes 7 of the 12 gene products produced by the 11 *met* regulon transcription units (1, 22, 26). These transcription units are organized into eight genetic loci on each organism’s chromosome. A negative regulatory system responsible for controlling expression of the *met* regulon is mediated by the *metJ* and *metK* genes which encode the regulon aporepressor and methionine adenosyltransferase (EC 2.5.1.6), respectively (9). The MetK protein catalyzes the formation of the corepressor molecule S-adenosylmethionine (AdoMet). The active-repressor negatively controls *met* regulon transcription units in a simultaneous but nonco-ordinate manner (28).

The *metJ* nucleotide sequence contains 106 codons which define a protein monomer with 104 amino acid residues (24). In its native form, MetJ exists as a dimer with a molecular weight of 24,000 (31). Each *E. coli* cell contains an estimated 600 copies of MetJ (23). Recently, Kafferty et al. (21) reported the structures of wild-type MetJ crystals formed in the presence or absence of AdoMet. The MetJ protein conformation is not changed by the binding of two corepressor molecules. Both crystal structures depict compact molecules in which two monomers form intersubunit contacts along adjacent faces of equivalent amino acid α-helices (the B-α-helix) and associated β-strands. Purified MetJ has been used in vitro to study *met* regulon expression and DNA footprinting patterns. Together, these studies demonstrated that in the presence of AdoMet, MetJ inhibited *met* regulon DNA-directed RNA synthesis and exhibited DNA sequence-specific binding within the *metJ/metBL*, *metC*, and *metF* promoter regions (20, 29, 30). The active MetJ dimer exhibits DNA sequence-specific binding to the consensus sequence AGACGTCT, which is referred to as the Met-box (4, 20). The pertinent DNA sequences of the *metA*, *metC*, *metF*, and *metJ/metBL* promoter regions contain from two to five imperfect, tandem repeats of the Met-box consensus sequence (3, 4, 7, 18, 24, 25). The homology of individual Met-box sequences varies significantly within and between *met* regulon elements (3, 4).

Recently we reported the isolation of the first temperature-sensitive *metJ* mutants (2). In complete medium, strains with one of these mutations, *metJ193*, repressed chromosomal *met* regulon elements at the permissive temperature of 28°C but lost regulatory ability at the nonpermissive temperature of 34 or 42°C. When the *metJ193* gene dosage was increased to two copies per chromosome, significant repression of both chromosomal and plasmid-borne *met* regulon elements occurred at the permissive temperature.

This article reports the nucleotide sequence of the *metJ193* allele and describes an additional regulatory dys-function of the MetJ193 protein: slow derepression of *met* regulon transcription units at the permissive temperature of 28°C. Nutritional downshift and maxi-cell experiments both provide evidence that the MetJ193 protein is aberrant in this respect. The kinetics of derepression of *met* regulon elements in the presence of the *metJ193* allele were also measured in temperature upshift experiments. The implications of these results are discussed in terms of the identified mutational lesion. This point mutation alters an integral component of the hydrophobic pocket which is the in vitro binding site for AdoMet in the crystallized wild-type MetJ protein (21).

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TABLE 1. Bacterial and bacteriophage strains

<table>
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<tr>
<th>Strain or phage</th>
<th>Relevant genotype</th>
<th>Plasmid* Reference</th>
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</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JJ116</td>
<td>Prototroph, metJ+ relAI</td>
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</tr>
<tr>
<td>JJ100F(O)</td>
<td>metJ+ pmetJ100 relAI</td>
<td>15</td>
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<tr>
<td>JJ117</td>
<td>Prototroph, metJ193</td>
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</tr>
<tr>
<td>JJ117.2</td>
<td>metJ193 metB1 relAI λ</td>
<td>2</td>
</tr>
<tr>
<td>JJ122R(pE3J-1B)</td>
<td>metJ193 metB1 relAI λ pmet100(J193)</td>
<td>eJE3-1B, eJE3-1B 8</td>
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<tr>
<td>GB950.1R(pE3J-1B)</td>
<td>metJ193 metF1 relAI recA58</td>
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<tr>
<td>Bacteriophages</td>
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<td>λ pmet100</td>
<td>c1857 Sam7, gal-type transducing phage which carries the E. coli metJ+ and metB+ alleles</td>
<td>15</td>
</tr>
<tr>
<td>λ pmet100(J193), isolates 8A and 5A</td>
<td>c1857 Sam7, gal-type transducing phage which carries the E. coli metJ193 and metB+ alleles</td>
<td>2</td>
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* Plasmid pE3J-1B contains the E. coli metF+ gene on a 6.7-kbp BamHI restriction fragment of lambda dmet128::Tn5[13] cloned into the single BamHI restriction site of pBR322 (8, 32).

MATERIALS AND METHODS

Strains, media, and chemicals. Bacteria and bacteriophages are described in Table 1. Luria-Bertani (LB) medium was used to propagate bacterial strains (19), and LB-lambda medium (12) was used to grow host cells for lambda phage propagation. Davis and Mingioli (6) salts with the omission of citrate (DM) were used to prepare minimal medium supplemented with 0.5% dextrose and, when appropriate, 200 μM methionine or 0.2% vitamin-assay-casamino acids (Difco Laboratories). When necessary, LB and DM media were supplemented with antibiotics. Reagent-grade chemicals were obtained from standard sources. Sequenase V 2.0 kits were purchased from the United States Biochemical Corporation, and [35S]dATP (specific activity, >1,000 Ci/mmole) was purchased from Amersham Corporation. Uniform 14C-labeled, methionine-free mixtures of 15 L-amino acids (284 mCi/m mole) were purchased from the Du Pont-New England Nuclear Division.

Nucleotide sequence analysis of the metJ193 allele. With minor modifications, dideoxynucleotide sequencing reactions were performed with lambda pmet100(J193) DNA templates prepared by the procedure of Manioli and Schneider (17). Oligonucleotide primers were synthesized on a Milligen 7500 DNA synthesizer by the protocols of the manufacturer. Primer pJ1 (ATCAACTGTCTTGCTGTCGTC) hybridizes to the coding strand and initiates DNA synthesis 2 base pairs (bp) upstream of the metJ start codon. Primer pJ3 (TAT CGCCGCTCCTATCCAGCATGATAC) hybridizes to the noncoding strand and initiates DNA synthesis 8 bp upstream of the metJ start codon. Primer pJ4 (TTTTT TGGTTIAGATTCC) hybridizes to the noncoding strand and initiates DNA synthesis at the 30th base of the metJ sequence. Lambda pmet100(J193) DNA (1 μg) was mixed with 100 ng of primer, boiled for 4 min, and quickly frozen in liquid nitrogen instead of dry ice. Hybridization was performed as the mixture thawed on ice. Sequencing reactions were performed with a Sequenase V 2.0 kit and [35S]dATP (17). Sequencing products were resolved on 6 or 8% polyacrylamide-Tris-borate-urea (pH 8.0) gels. The resulting electrophorograms were dried under vacuum at 60°C and used to prepare autoradiograms (27). DNA sequences were read manually from autoradiograms.

Growth strains for temperature upshift studies. Each strain was grown in LB medium at 28°C for 12 to 16 h. A portion of the LB culture was diluted in DM medium supplemented with 0.5% dextrose and 200 μM methionine and incubated at 28°C for 12 to 13 h. The overnight culture was diluted 20-fold into duplicate 1.1-liter volumes of equivalent medium and incubated in a New Brunswick model G25 air incubator at 28°C. After 4 h, time zero samples (40 OD650 units) were removed (half of the volume needed from each duplicate flask) for quantitative enzyme assays. One of the duplicate 1.1-liter cultures was placed in a reciprocating water bath at 34°C for 30 min to achieve rapid temperature equilibration. Following the initial 30 min after temperature shift, the 34°C culture was incubated in a second New Brunswick model G25 air incubator at 34°C. Spectrophotometric readings and samples (40 OD650 units) were taken from both the 28 and 34°C cultures at 15, 30, 45, 60, 120, and 180 min after temperature induction. The samples were prepared for enzyme assays as described previously (2, 10).

Growth strains for nutritional downshift studies. Strains were grown and conditioned in DM medium supplemented with dextrose and methionine as described for the temperature upshift experiments. Two 1-liter volumes of the medium were inoculated and incubated at 28°C until they reached an approximate OD650 of 0.6. Timed, −1 h (40 OD650 units) samples were removed (half of the volume drawn from each duplicate culture) for enzyme assays. The combined cultures were washed once by centrifugation and suspension in DM salts at 28°C. Half of the washed cells were suspended in 25 ml of DM plus dextrose and methionine, and the other half were suspended in an equivalent volume of DM medium with 0.5% dextrose. One fresh, warm liter of each medium was inoculated with the appropriate amount of washed, resuspended cells. The time of inoculation was designated time zero of the nutritional downshift. Spectrophotometric readings and samples (40 OD650 units) were taken from both cultures at 0, 15, 30, 45, 60, 90, 120, and 180 min after nutritional downshift (for strain JJ117.2 samples were also harvested at 4, 5, and 6 h after downshift). The samples were prepared for enzyme assays as described previously (2, 10).

Growth strains and preparation of maxicell samples. Cultures of metJ+ or metJ193, recombination-deficient (recA58 [16]) strains bearing plasmid pE3J-1B (Table 1) were grown at 28°C in DM medium containing dextrose, casamino acids, and appropriate antibiotics. Maxicell samples were prepared from these cultures by UV irradiation as described by Emmett and Johnson (8). Before 14C-amino acids were used to label protein products, the exogenous methionine concentrations of these maxicell samples were manipulated as described in the Results (8). According to published procedures, the labeled samples were harvested, processed, resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and the resulting Coomassie blue-stained electropherogam was analyzed by autoradiography (2, 8, 13, 14).
RESULTS

Nucleotide sequence analysis of the metJ193 allele. The protocol of Manfioletti and Schneider (17) was used to determine the metJ193 nucleotide sequence (Materials and Methods). Appropriate regions of intact chromosomal DNA separately isolated from viral samples of two isogenic lambda pmet100(JJ193) transducing phage strains (Table 1) were sequenced (data not shown). One 30-base and two 20-base oligonucleotides complementary to DNA sequences either upstream of the 5' end (pJ1 and pJ3) or overlapping the 3' end (pJ4) of the reported wild-type metJ sequence (24) were used as primers for sequencing reactions (Materials and Methods). The pJ1 primer was used to determine the complete sequence of the noncoding strand of the metJ193 allele carried by both lambda pmet100(JJ193) isolates, and the pJ4 primer was used to sequence a portion of the metJ193 coding strand defining the protein's amino terminus plus part of the upstream sequence encoding the three metJ promoters (11). The remainder of the metJ promoter sequence was determined by using the pJ3 primer.

Comparison of the metJ193 and wild-type metJ (24) nucleotide sequences revealed a single point mutation, a T to A transversion, at the bp 170 of the metJ193 open reading frame (Fig. 1). This point mutation altered the 56th residue of the MetJ193 amino acid sequence, replacing leucine with glutamine (Fig. 1). No mutations were found in the promoter or operator sequences of the metJ193 allele (data not shown). Based upon the MetJ crystal structure, amino acid residues 52 to 66 form the B-α-helix which is important to the dimer structure of the protein and in the binding of AdoMet (21).

Effects of a temperature upshift on cellular growth and expression of met regulon enzymes. At the permissive temperature, 28°C, cultures of JJ116, JJ117, and JJ117.2 (Table 1) were grown in DM medium supplemented with dextrose and methionine. Half of each mid-log-phase culture was shifted to the nonpermissive temperature, 34°C, and the growth of each was monitored (Materials and Methods) (Fig. 2, column A). The specific activities of cystathionine-γ- synthetase (metB) and β-cystathionase (metC) were measured in extracts prepared from samples taken at timed intervals following division of the cultures and the temperature upshift. Repressed levels of enzyme specific activity were observed in all the culture samples which were maintained at the permissive temperature (Fig. 2, columns B and C, open symbols). Between 0.5 and 2.0 h at 34°C, cultures of strains JJ117 (one metJ193 allele) and JJ117.2 (two metJ193 alleles) exhibited significant derepression of metB and metC expression (Fig. 2, columns B and C, solid symbols, JJ117 and JJ117.2 panels). Compared with the JJ117 34°C culture, lower specific activities of both enzymes were found in cell extracts prepared from the JJ117.2 34°C culture (Fig. 2, columns B and C, JJ117 and JJ117.2 panels).

Effects at 28°C of a nutritional downshift on growth and expression of met regulon enzymes. Strains JJ116, JJ100F[O], JJ117, and JJ117.2 (Table 1) were grown to mid-log phase at 28°C in flasks of DM medium supplemented with dextrose and methionine. Cells were harvested and either maintained in minimal medium containing methionine or nutritionally downshifted to minimal medium as described in Materials and Methods. Typical patterns of growth in the presence or absence of exogenous methionine were observed for strains JJ116, JJ100F[O], and JJ117. However, the culture of strain JJ117.2 which was nutritionally downshifted exhibited a significant growth lag (Fig. 3, column A). The specific activities of cystathionine-γ-synthetase and β-cystathionase were measured for samples taken from each culture at timed intervals after nutritional downshift (Materials and Methods) (Fig. 3, columns B and C). Repressed levels of enzyme specific activity were observed for all samples from cultures maintained in DM medium supplemented with dextrose and methionine. Immediate (0.25 h) increases in cystathionine-γ-synthetase and β-cystathionase specific activities were observed in the first temporal samples taken from the JJ116 and JJ100F[O] cultures which were transferred to DM-dextrose medium. Enzyme specific activities in these cultures plateaued by 0.75 h after the nutritional downshift (Fig. 3, columns B and C, JJ116 and JJ100F[O] panels).
MetJ and MetJ193 proteins between apo- and active-repressor states were compared with a maxicell system (8) to measure regulation of the plasmid pEJ3-1B-borne metF transcription unit (2, 8) (Materials and Methods). Under the growth conditions used to prepare the starting cell cultures, strains with one copy of metJ (JJ122R[pEJ3-1B]) or two copies of metJ193 [GB950.1R(pEJ3-1B)] maintained repression of the plasmid-borne metF gene, but strains with one metJ193 allele [GB950.1R(pEJ3-1B)] produced constitutive levels of MetF protein (2).

From each culture, two UV-irradiated maxicell samples, one in DM medium containing dextrose plus antibiotics (− sample) and one in the same medium supplemented with exogenous methionine (+ sample), were prepared and incubated at 28°C (Materials and Methods). After 3.5 h, each sample was again subdivided into two portions which were incubated in medium unsupplemented or supplemented with exogenous methionine (−/+ and +/−) and (+/+) samples, respectively. After an additional 30 min at 28°C, 6 μCi of methionine-free 14C-amino acids was added to each sample. At 4.5 h after UV irradiation, the radiolabeled samples were processed and analyzed by SDS-PAGE as described in Materials and Methods. An autoradiograph prepared from the resulting electropherogram revealed that during the 30-min 14C-labeling period, gene product MetF synthesis was constitutive in the JJ122R[pEJ3-1B] +/− and −/− samples (Fig. 4, right panel, lanes 2 and 4) and repressed in the +/+ and −/+ samples (Fig. 4, right panel, lanes 1 and 3). The Comassie blue-stained electropherogram revealed that unlabeled gene product MetF was synthesized only in the JJ122R[pEJ3-1B] −/+ sample (Fig. 4, both panels, lane 3). Thus, the transitions of gene product MetJ between apo- and active repressor were reversible in 30 min or less. All the GB950.1R[pEJ3-1B] samples produced intense gene product MetF bands on both the electropherogram and autoradiogram (Fig. 4, both panels, lanes 5 to 8) (2). The GB950.2R[pEJ3-1B] samples did not exhibit gene product MetF bands on the stained electropherogram (Fig. 4, left panel, lanes 9 to 12), and only small amounts of gene product MetF were visible on the autoradiogram (Fig. 4, right panel, lanes 9 to 12). Therefore, in the absence of exogenous methionine, two gene doses of gene product MetJ193 repressed expression of a plasmid-borne metF allele throughout the labeling period, and in the presence or absence of exogenous methionine one gene dose of gene product MetJ193 could not regulate the plasmid-borne metF allele.

**DISCUSSION**

The recently reported crystal structure of the MetJ protein complex with AdoMet (21) provides a context for interpretation of the metJ193 mutation. The T to A transversion at the 170th base of the metJ193 open reading frame converts the fifth amino acid residue of the B-α-helix (residues 52 to 66) from leucine to glutamine (Fig. 1). This mutational lesion modifies one of the amino acid side chains (residue 56) which Rafferty et al. (21) reported constitute the hydrophobic pocket where in vitro binding of the AdoMet corepressor occurs (residues 70, 56, 59, 43, 63, 64, 61, and 65). Visual inspection of the three-dimensional structure of the hydrophobic pocket suggests that there are several possible effects on the MetJ193 protein structure which could result from the glutamine 56 substitution. All of these proposed effects would occur due to the increased size and hydrogen-bonding capacity of the glutamine side chain. The described amino
FIG. 3. Effects of a nutritional downshift on strain growth and the induction of cystathionine-γ-synthetase (metB) and β-cystathionase (metC) in cultures of strains JJ116, JJ100F(0), JJ117, and JJ117.2 (Table 1). The panels in column A depict culture growth following nutritional downshift at 28°C in DM medium containing 0.5% dextrose supplemented (open symbols) or unsupplemented (solid symbols) with 200 μM methionine. Panels in columns B and C represent the specific activities (nanomoles of product per minute per milligram of protein) of cystathionine-γ-synthetase (column B) and β-cystathionase (column C) present in cell extracts prepared from samples harvested at the indicated time points after nutritional downshift. In each panel the open symbols represent the specific activities present in cultures maintained in medium supplemented with methionine, and the solid symbols represent similar values for cultures shifted to unsupplemented minimal medium. The nomenclature in the upper left-hand corner of each panel indicates the identity of the strain.

The structure of the B-α-helix is proposed to be important for proper assembly and stability of the MetJ protein dimer (21). The position of the mutational lesion within the B-α-helix (which is involved in intersubunit contacts in the wild-type protein dimer) obviously confers instability to the effects of temperature on the MetJ193 protein’s repressor activity. The physical interactions underlying this instability are unknown, but the amino acid substitution could possibly decrease intersubunit hydrophobic interactions, thereby increasing the thermolability of the protein dimer (5, 21).

The temperature upshift experiment revealed that a slow transition (approximately 30 min, Fig. 2) from repression to derepression of met regulon elements occurred in strains bearing the metJ193 allele. Several factors could contribute to this phenomenon. The MetJ193 protein dimers assembled at 28 or 34°C may assume different structural conformations which directly influence their ability to repress met regulon...
expression. However, the MetJ193 protein formed at 28°C may retain its function at 34°C. In the latter case, the observed derepression would require dilution of the functional 28°C repressor through cell growth and division at 34°C, where only defective MetJ193 protein would be produced. Alternatively, the MetJ193 protein may have similar structure at both 28 and 34°C but may interact differently with met regulon operators at the two temperatures.

Following the shift from 28 to 34°C, no effect of metJ193 gene dose on the kinetics of met regulon derepression occurred, but an effect on the level of derepression was observed (Fig. 2). However, the nutritional downshift studies demonstrated that under appropriate conditions of gene dose, the MetJ193 protein does not allow derepression of met regulon expression to occur quickly enough to avoid a slowing of strain growth (Fig. 3). This growth delay, or lag, is apparently due to methionine deprivation resulting from derepression of met regulon expression. Recovery of growth coincides with derepression of met regulon elements, which suggests that the ability of the wild-type MetJ protein to move quickly between the active-repressor and aporepressor forms is extremely important for maintenance of a proper relationship between met regulon expression and cell growth requirements. The maxicell studies of the expression of an amplified plasmid-borne metJ193 allele support both the increased repression effect of metJ193 gene dose on met regulon elements and the inability of the MetJ193 protein to accomplish transition between the aporepressor conformations as easily as the wild-type protein. The lesion found to exist in the metJ193 allele suggests that the growth defect results from the altered structure of the MetJ193 protein and not from an alteration in the promoter/operator region of the gene (Fig. 1 and Results).

In summary, our results confirm the importance of the recently described B-a-helix and the associated in vitro AdoMet-binding site regions to the in vivo function of the MetJ protein as the metJ regulon repressor. Current studies in our laboratory are investigating met regulon transcription patterns in the presence of the MetJ193 protein and the in vivo as well as in vitro recognition and interaction of this mutant protein with met regulon operator sequences.

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LITERATURE CITED


