Cloning and nucleotide sequence of the *Salmonella typhimurium* LT2 metF gene and its homology with the corresponding sequence of *Escherichia coli*

George V. Stauffer and Lorraine T. Stauffer

Department of Microbiology, University of Iowa, Iowa City, IA 52242, USA

Summary. The *Salmonella typhimurium* LT2 metF gene, encoding 5,10-methylenetetrahydrofolate reductase, has been cloned. Strains with multicopy plasmids carrying the metF gene overproduce the enzyme 44-fold. The nucleotide sequence of the metF gene was determined, and an open reading frame of 888 nucleotides was identified. The polypeptide deduced from the DNA sequence contains 296 amino acids and has a molecular weight of 33135 daltons. Mung bean nuclease mapping experiments located the transcription start point and possible transcription termination region. This region possesses a GC-rich sequence that could form a stable stem and loop structure once transcribed (ΔG = -9 kcal/mol), followed by an AT-rich sequence, both of which are characteristic of rho-independent transcription terminators. The nucleotide and deduced amino acid sequences of the *S. typhimurium* metF gene are compared with the corresponding sequences of the *Escherichia coli* metF gene. The nucleotide sequences show 85% homology. Most of the nucleotide differences found do not alter the amino acid sequences, which show 95% homology. The results also show that a change has occurred in the metF region of the *S. typhimurium* chromosome as compared to the *E. coli* chromosome.

**Key words:** metF – 5,10-methylenetetrahydrofolate reductase – Recombinant DNA – Nucleotide sequence

**Introduction**

In *Salmonella typhimurium* and *Escherichia coli*, the methylation of homocysteine to form methionine is catalyzed by either of two transmethylase enzymes. One, the metH-encoded transmethylase, is vitamin B₁₂-dependent, whereas the other, the metE-encoded transmethylase, is vitamin B₁₂-independent (for a review, see Rowbury 1983). The methyl group transferred by these enzymes is donated by 5-methylTHF, which is produced by the metF gene product, 5,10-methylenetetrahydrofolate reductase.

**Abbreviations:** 5-methylTHF, 5-methyltetrahydrofolate; 5, 10-methylenetetrahydrofolate; (), designates plasmid-carrier state; Tc, tetracycline; GM, glucose minimal; Ap, ampicillin; ORF, open reading frame

**Offprint requests to:** G.V. Stauffer

The metF gene, as well as all of the other methionine biosynthetic genes except metH, is negatively controlled by the metJ gene product, with S-adenosylmethionine acting as the co-repressor (Greene et al. 1973; Shoeman et al. 1985; Emmett and Johnson 1986). In addition, metF, as well as metE, is negatively controlled by the metH gene product, with vitamin B₁₂ acting as the co-repressor (Milner et al. 1969; Greene et al. 1973). This latter regulatory system, although distinct from the metJ regulatory system, requires a functional metJ gene product (Dawes and Foster 1971; Kung et al. 1972). In contrast, the metF regulatory system functions independently of the metH regulatory system to control expression of metF and the other met genes. In addition, a functional metF gene is required for the vitamin B₁₂-mediated control of the metE and metF genes (Mulligan et al. 1982).

The metF gene has been cloned from *E. coli*, its nucleotide sequence has been determined, and several mutations have been identified that locate the MetJ repressor binding site (Saint-Girons et al. 1983; Belfaïza et al. 1987). We have cloned the metF gene from *S. typhimurium* and have begun to characterize the gene by using genetic and biochemical techniques. Here we present the complete nucleotide sequence of the *S. typhimurium metF* gene and compare it with the corresponding sequence from *E. coli*.

**Materials and methods**

**Bacterial strains and plasmids.** *S. typhimurium* strain JL781 is wild type (Stauffer and Brenchley 1977) and was used for the preparation of chromosomal DNA, and strain JB672 is metI (Stauffer and Brenchley 1977) and was used for the preparation of total cellular RNA. *E. coli* strains GS232 (metF63, pro-22) and GS236 (hsdR4, endA, sbc15, thi) were from C. Yanofsky. *E. coli* strain GS162 is AlacU169, pheA905, araD139, rpsL, thi (Stauffer et al. 1981) and strain GS687 is AlacU169, pheA905, araD139, rpsL, thi, ΔmetF::Mu (this laboratory). Plasmid vectors used are pBR322 (Bolivar et al. 1977) and pACYC184 (Chang and Cohen 1978). Plasmids pGS139 and pGS174 carry the S. *typhimurium metF* gene and were constructed during this investigation.

**Media, growth of cells and extract preparation.** Media, growth of cells and preparation of extracts have been described previously (Stauffer et al. 1981). Antibiotics were
added at the following concentrations: 150 μg/ml ampicillin; 10 μg/ml tetracycline. Amino acids were added at 50 μg/ml and vitamins were added at 1 μg/ml.

Enzyme assays. The 5,10-methylenetetrahydrofolate reductase (metF gene product) was assayed as described by Kutzbach and Stokstad (1971). Protein determinations were made by the method of Lowry et al. (1951).

DNA isolation and manipulation. S. typhimurium chromosomal DNA was isolated as described previously (Stauffer et al. 1981). Restriction enzyme digestion, DNA ligation, transformation and agarose and polyacrylamide gel electrophoresis are as described in Maniatis et al. (1982). The DNA sequencing method was that of Maxam and Gilbert (1980). Gel electrophoresis was according to Sanger and Coulson (1978).

5' and 3' mung bean nuclease mapping. The S1 nuclease mapping procedure of Weaver and Weissmann (1979) was used with slight modification. Mung bean nuclease and mung bean buffer (30 mM sodium acetate pH 4.6, 50 mM sodium chloride, 1 mM zinc chloride and 5% glycerol) were used in place of S1 nuclease and S1 buffer. To determine the transcription initiation site, a 466 bp HindIII-BstEII DNA fragment deduced to contain the metF transcription initiation site was labeled at the 5' ends with γ-[32P]ATP using T4 polynucleotide kinase (Maniatis et al. 1982). The labeled strands were separated and purified electrophoretically (Maxam and Gilbert 1980). An aliquot of each strand was hybridized to about 25 μg of total cellular RNA isolated from strain JB672(pGS174). The RNA-DNA hybrids were digested with either 30 or 100 units of mung bean nuclease and ampicillin from strain JB672(pGS174). The RNA-DNA hybrids were hybridized to about 25 μg of total cellular RNA isolated (Maxam and Gilbert 1980). An aliquot of each strand was then treated as in the 5' mapping procedure described above.

Enzymes and chemicals. All enzymes were from NEB (Beverly, Mass), BRL (Gaithersburg, Md) and Pharmacia (Piscataway, NJ). Radioisotopes were from Amersham (Arlington Heights, Ill). All other chemicals were reagent grade and commercially available.

Results and discussion

Cloning the S. typhimurium metF gene

The DNA fragments from a partial EcoRI digest of S. typhimurium chromosomal DNA (10 μg) were ligated into the EcoRI site of the plasmid vector pACYC184 (5 μg) and the ligation mixture was used to transform the broth (Fig. 1). The DNA fragments from a partial EcoRI digest of S. typhimurium chromosomal DNA (10 μg) were ligated into the EcoRI site of the plasmid vector pACYC184 (5 μg) and the ligation mixture was used to transform the restriction-negative modification-positive E. coli strain GS236 with selection on L-agar plates plus Tc since pACYC184 confers Tc resistance. All Tc° colonies were harvested and plasmid DNA was prepared. The plasmid DNA was used to transform E. coli strain GS687 (AmetF::Mu) with selection on GM plates supplemented with phenylalanine, thiamine and Tc. One Met° Tc° transformant was isolated. Plasmid DNA isolated from this strain was designated pGS139. Restriction enzyme analysis showed that plasmid pGS139 contains two EcoRI insert fragments of about 7.4 and 1.0 kb (Fig. 1).

Subcloning of metF

A comparison of the physical map of plasmid pGS139 with that of the E. coli metF gene (Saint-Girons et al. 1983) suggested that the S. typhimurium metF gene is located on a 4.3 kb SalI-ClaI DNA fragment (Fig. 1). DNA fragments resulting from a SalI plus ClaI digestion of plasmid pGS139 were ligated into the SalI and ClaI sites of plasmid pBR322. The ligation mixture was used to transform the E. coli metF mutant strain GS687, selecting for methionine prototrophy on GM plates supplemented with phenylalanine, thiamine and ampicillin since plasmid pBR322 confers Ap resistance.