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NOTES

Nucleotide Sequence of the *Salmonella typhimurium mutB* Gene, the Homolog of *Escherichia coli mutY*

VARALAKSHMI DESIRAJU,[1]† WILLIAM G. SHANABRUCH,[2] AND A-LIEN LU[1]*

Department of Biological Chemistry, University of Maryland, Baltimore, Maryland 21201,[1] and Department of Biology, University of Richmond, Richmond, Virginia 23173[2]

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The *mutB* gene of *Salmonella typhimurium* is involved in a methylation-independent repair pathway specific for A/G or A/C mismatches and is the homolog of the *Escherichia coli mutY* gene. The *mutB* gene of *S. typhimurium* was cloned and sequenced. The isolated *mutB* clone reduced the mutation rate of the *mutB* mutant to wild-type levels and also restored A/G mismatch-specific nicking activity, which is defective in *mutB* extracts. The amino acid sequence encoded by the *mutB* gene is 91% homologous to that encoded by the *E. coli mutY* gene.

Several repair pathways for DNA base mismatches have been found in *Escherichia coli* and *Salmonella typhimurium*. A repair system which is not controlled by *dam* methylation (4, 16) and is dependent on the *mutY* gene product (1, 12, 13, 18) has been identified in *E. coli*. This pathway is specific for conversion of A/G or A/C mismatches to C · G or G · C base pairs, respectively (1, 4, 5, 13, 16). However, A/C repair activity cannot be detected in wild-type extract in vitro (4, 16) and the nicking activity of MutY on an A/C substrate is 20-fold lower than that on an A/G substrate (17). Recently, it has been shown that MutY can also act on adenines mispaired with 7,8-dihydro-8-oxo-guanine (8). Miller and coworkers suggested that the function of MutY is to remove the misincorporated adenines from A/7,8-dihydro-8-oxo-guanine mispairs (8, 9). The MutY protein has been purified and shown to be an adenine glycosylase (2) and subsequently shown to have an associated apurinic endonuclease activity (17). The DNA glycosylase removes the adenine base at the A/G, A/C, or A/7,8-dihydro-8-oxo-guanine mismatch, and the apurinic endonuclease cleaves the first phosphodiester bond 3′ to the apurinic site.

It has been suggested that the *mutB* gene of *S. typhimurium* is the homolog of *E. coli mutY* (6). The *mutB* mutant exhibits a higher rate of C · G-to-A · T transversions than wild-type cells and has no effects on other transversion or transition events (6, 15). The *mutB* mutant extract is defective in A/G-specific binding and nicking and repair activities (6).

Here we report the cloning and nucleotide sequencing of the *mutB* gene. The protein sequence predicted by the DNA sequence of *mutB* is highly homologous to that of the *E. coli* MutY protein. The *mutB* gene was cloned on the basis of its ability to decrease the mutation rate of *mutB* mutants and restore A/G mismatch-specific nicking activity to *mutB* mutant extracts.

* Corresponding author.

† Present address: Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80262.

Cloning of the *mutB* gene of *S. typhimurium*. Strain GW1803 (*hisG46 gal-6mutB131*::Tn*5*) contains a Tn*5* insertion at the *mutB* gene (15). The kanamycin resistance gene (*kan*) of Tn*5* provided a tag to clone the *mutB* gene. Chromosomal DNA prepared from GW1803 (*mutB*::Tn*5*) was digested with *Sal*I (which does not cleave the *kan* gene) and ligated to *Sal*I-digested vetor pUC8. Transformants resistant to both kanamycin (Kan^r) and ampicillin (Amp^r) were selected. Plasmid pWS304 (Fig. 1) contained an 11.4-kb DNA insert of which 2.7 kb is derived from Tn*5* DNA (the left inverted repeat and the *kan* gene). The 8.7 kb of *Salmonella* chromosomal DNA flanking Tn*5* in pWS304 was presumed to contain part of the *mutB* transcriptional unit.

The 1.1-kb *Eco*RI fragment close to the Tn*5* DNA from pWS304 (Fig. 1) was used as a probe to identify the intact *mutB* gene from an *S. typhimurium* library (19). The library (a generous gift from Masahiko Watanabe) contained partial *Sau*3AI digests of *S. typhimurium* TA1538 (*hisD3052*) genomic DNA cloned into the *Bam*HI site of pBR322 (19). About 5,000 Amp^r transformants were screened by hybridization with the 1.1-kb *Eco*RI probe from pWS304 as described by Maniatis et al. (7). Of 11 positive candidates, two clones designated pVV85 (with a 5.5-kb insert) and pVV149 (with a 9.5-kb insert) were selected for analysis.

Complementation of the *S. typhimurium mutB* mutant phenotype by the cloned *mutB* gene. To identify an intact *mutB* gene, both pVV85 and pVV149 were transformed into *mutB* cells to test their ability to complement the *mutB* mutation. The mutator phenotype of the *mutB* mutant was completely suppressed by pVV149 but not by pVV85 (Fig. 2). Furthermore, both pVV85 and pVV149 were tested for the ability to complement *mutB* mutant extracts by restoring A/G-specific nicking activity. Plasmid pVV85, pVV149, or pBR322 was transformed into GW1803; cell extracts were prepared from these strains and tested for A/G nicking activities by the method described by Lu et al. (6). Plasmid pVV149, but not pVV85 or pBR322, was able to complement the *mutB* mutation in this assay (Fig. 3). Therefore, pVV149 contained a functional *mutB* gene that could be transcribed and translated to complement the *mutB* mutation. Plasmid pVV85 was shown to contain a truncated *mutB* gene (data not shown).

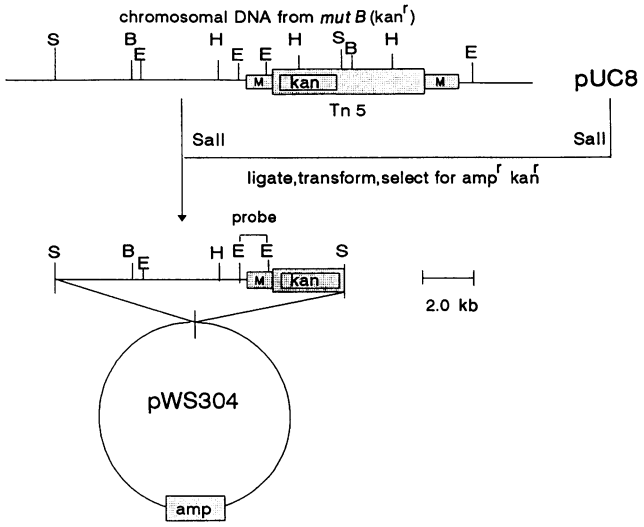

FIG. 1. Construction of a plasmid containing part of the *mutB* gene from GW1803 (*mutB*::Tn*5*) genomic DNA. Genomic DNA from *mutB*::Tn*5* mutant GW1803 (shown on the top) was isolated, digested with *Sal*I, and cloned into the *Sal*I site of vector pUC8. The boxed region represents the *mutB* gene (M) with the Tn*5* insertion in a larger box. A clone designated pWS304, resistant to both ampicillin (amp) and kanamycin (kan) was selected. The insert in pWS304 (11.4 kb) from GW1803 contains part of Tn*5*, as well as *S. typhimurium* chromosomal DNA. The 1.1-kb *Eco*RI fragment that flanks the *kan* gene of Tn*5* was used as a probe to screen an *S. typhimurium* gene library for the wild-type *mutB* gene. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I.

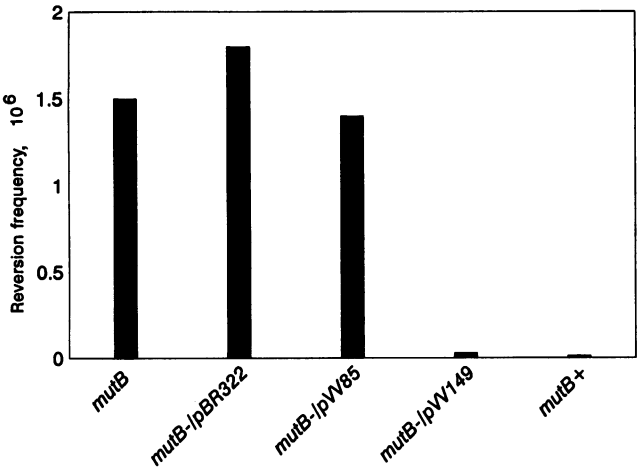

FIG. 2. Complementation of *mutB* mutation by pVV85 and pVV149. GW1803 *mutB* mutant cells were transformed with plasmid pBR322, pVV85, or pVV149, and mutation frequencies were measured. The mutation frequency is given as the ratio of the number of rifampin-resistant colonies to the number of viable cells obtained by plating overnight cultures on LB medium (11) with rifampin (0.1 mg/ml) or suitable dilutions on LB plates, respectively. The *mutB*$^+$ wild-type cell was GW2 (*hisG46 gal-6*) (15).

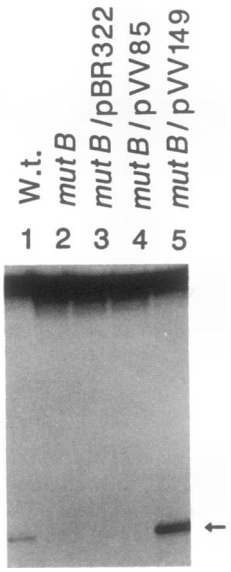

FIG. 3. Complementation of A/G-specific nicking in extracts of *mutB* cells containing *mutB* plasmids. GW1803 *mutB* mutant cells (lane 2) were transformed with plasmid pBR322 (lane 3), pVV85 (lane 4), or pVV149 (lane 5). Extracts were prepared from these cells and a nicking assay to A/G-containing DNA (with 3′-end labeling on the dA strand) was performed as previously described (6). The *mutB*$^+$ wild-type (w.t.) cell extract (lane 1) was from GW2 (*hisG46 gal-6*) (15). The arrow indicates the cleavage product of MutB protein.

With the extract from *S. typhimurium* LB 5010 (*ilv leu met trp galE* r$^-$ m$^+$) containing plasmid pVV149, we tested nicking activity on A/C-containing DNA. The MutB protein, overexpressed in these cells owing to the high copy number of the plasmid, had weak nicking activity on A/C substrates (data not shown). MutB nicking activity on A/C-containing DNA was about 10% of that on the A/G substrate. This weak A/C nicking activity was not detected with extracts from wild-type cells (data not shown). This is consistent with the results obtained with MutY from *E. coli*. *E. coli mutY*-dependent A/C repair was shown by an in vivo assay (13) but could not be detected in a wild-type extract in vitro (4, 16). When MutY was overproduced, weak nicking on A/C substrates was detected with an excess of the MutY enzyme (17).

Nucleotide sequence of the *S. typhimurium mutB* gene. The nucleotide sequence of the *mutB* gene was determined (Fig. 4) by the dideoxy-chain termination method (14) by using the Sequenase kit (United States Biochemical Corp., Cleveland, Ohio). First, a 20-nucleotide oligonucleotide (5′CGTTCAG GACGCTACTTGTG3′) identical to the 5′ end of the inverted repeat of Tn*5* was used as a primer to sequence the junction between the chromosomal DNA and Tn*5* of pWS304. The sequence information obtained was used to design other primers to "walk" across the *mutB* gene on both DNA strands of pVV149. The nucleotide sequence reported here has been deposited in the GenBank data base under accession number M86634. An open reading frame for 350 amino acids corresponding to a protein of 39.4 kDa was found in this sequence. Potential −35 and −10 sequences (3) are indicated in Fig. 4. This open reading frame was the *mutB* gene because: (i) plasmid pVV149 containing this complete open reading frame could complement the *mutB* mutation, (ii) a Tn*5* insertion within this open reading frame inactivated the *mutB* function, and (iii) its amino acid sequence was highly homologous to the *E. coli* MutY sequence.

In the MutB and MutY proteins, 91% of the amino acids are identical (Fig. 4). Among the nonidentical amino acids,

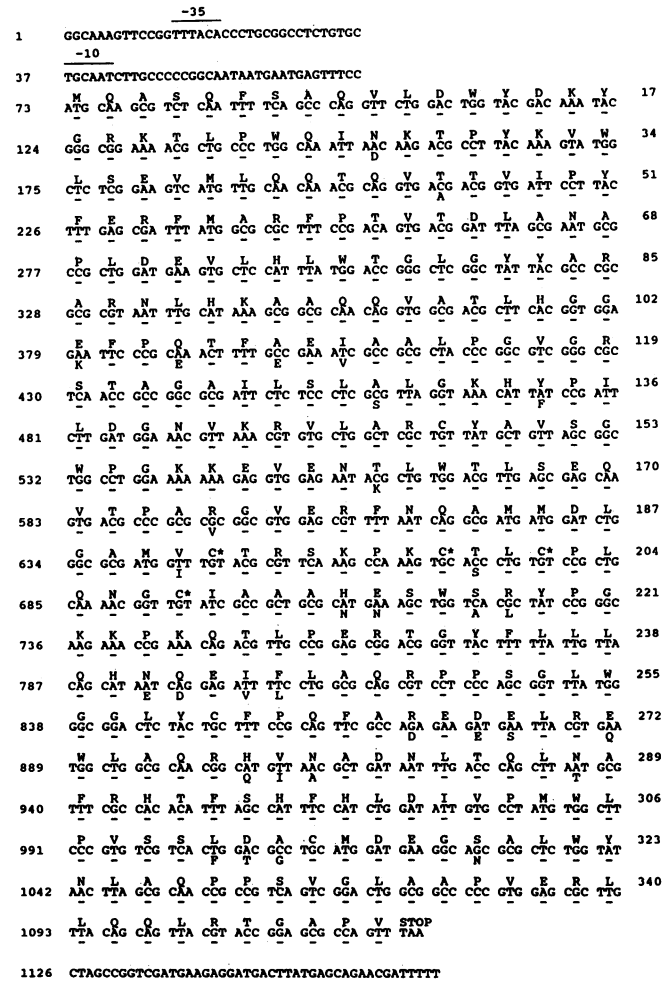

FIG. 4. Nucleotide sequence of the *S. typhimurium mutB* gene and predicted amino acid sequence of the MutB protein. The predicted amino acid sequences of MutB and MutY are shown above and below the *mutB* nucleotide sequence, respectively. The amino acids of MutY that are identical to those of MutB are shown as dashes. The four conserved cysteine residues that may bind to the $[4Fe\text{-}4S]^{2+}$ cluster are shown with asterisks. Potential −35 and −10 sequences (3) are indicated.

about 80% are similar. Moreover, the four cysteines (Cys-N_6-Cys-N_2-Cys-N_5-Cys) are conserved between the MutB and MutY proteins. Michaels et al. (10) found that *E. coli* MutY has homology to *E. coli* endonuclease III, which is an iron-sulfur protein. Recently, chemical and physical analyses have shown that MutY has a $[4Fe\text{-}4S]^{2+}$ cluster (6a). It is believed that the four conserved cysteines may be involved in binding to the $[4Fe\text{-}4S]^{2+}$ cluster. On the basis of the high degree of homology between *E. coli* MutY and *S. typhimurium* MutB, it is likely that *S. typhimurium* MutB is an iron-sulfur protein with both DNA glycosylase and apurinic endonuclease activities.

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