NOTES

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

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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 co-workers 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 adenosine 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 the *S. typhimurium* 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.

Cloning of the mutB gene of *S. typhimurium*. Strain GW1803 (hisG46 gal-bmutB131::Tn5) contains a Tn5 insertion at the mutB gene (15). The kanamycin resistance gene (*kan*) of Tn5 provided a tag to clone the mutB gene. Chromosomal DNA prepared from GW1803 (mutB::Tn5) was digested with *Sai*I (which does not cleave the *kan* gene) and ligated to *Sai*I-digested vector pUC8. Transformants resistant to both kanamycin (Kan') and ampicillin (*Amp') were selected. Plasmid pWS304 (Fig. 1) contained an 11.4-kb DNA insert of which 2.7 kb is derived from Tn5 DNA (the left inverted repeat and the *kan* gene). The 8.7 kb of *Salmonella* chromosomal DNA flanking Tn5 in pWS304 was presumed to contain part of the mutB transcriptional unit.

The 1.1-kb *EcoRI* fragment close to the Tn5 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 *San*AI and *Nco*I digests of *S. typhimurium* TA1538 (*hisD3052*) genomic DNA cloned into the *BamHI* site of pBR322 (19). About 5,000 *Amp* resistant transformants were screened by hybridization with the 1.1-kb *EcoRI* 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).

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With the extract from *S. typhimurium* LB 5010 (ilv leu met trp gal E 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'CGTTTAGCAGCGTACTTGG3') identical to the 5' end of the inverted repeat of Tn5 was used as a primer to sequence the junction between the chromosomal DNA and Tn5 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 Tn5 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,
about 80% are similar. Moreover, the four cysteines (Cys-N2-Cys-N2-Cys-N2-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-4S]2+ cluster (6a). It is believed that the four conserved cysteines may be involved in binding to the [4Fe-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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