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Cloning and Sequencing of *Escherichia coli mutR* Shows Its Identity to *topB*, Encoding Topoisomerase III

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We have cloned and sequenced the *mutR* gene from *Escherichia coli*, which results in an increased frequency of spontaneous deletions, by using a strain carrying a Tn*10* derivative inserted into *mutR*. The analysis of 1,286 bp of *mutR* sequence shows that this gene is identical to the *topB* gene, which encodes topoisomerase III. The increased deletion formation is the first reported phenotype for cells lacking topoisomerase III, and this suggests that topoisomerase III is involved in reactions that normally reduce the levels of spontaneous deletions.

We have previously described a locus, *mutR*, at 38.5 min on the genetic map of *Escherichia coli*, which affects deletion formation (10). Point mutations or insertions in *mutR* result in an increase in spontaneous deletions at short homologies (10). Because the pathways that lead to spontaneous deletions have not been completely defined, it is important to characterize the genes and gene products involved. In this paper, we report the cloning and sequencing of the *mutR* gene and show that it is identical to the *topB* gene, which also maps at 38.5 min and which encodes topoisomerase III, a type I topoisomerase (2).

Strategy to clone the *mutR* locus. To facilitate the cloning of the wild-type *mutR* locus, we used Southern (7) analysis to determine which of the four Kohara (4) *E. coli* lambda clones in the region near 38.5 min (λ327 to λ330) was the best candidate for carrying *mutR*. We used strain DB100, a derivative of strain DB1 (10) carrying a mini-Tn*10* (9) in the *mutR* gene (10). The chromosomal DNA of DB100 was digested with *Eco*RI or *Hin*dIII, either of which cuts once within the mini-Tn*10*, or *Pst*I, which does not cut the mini-Tn*10*. The digested DNA was electrophoresed through a 1% agarose gel in TAE (Tris-acetate-EDTA) buffer and hybridized to a radiolabeled oligonucleotide homologous to the ends of Tn*10*. The Tn*10* probe detected two *Eco*RI fragments of approximately 3.5 and >23 kb, a single 9-kb *Hin*dIII fragment, which we suspected was a doublet, and a single *Pst*I fragment of approximately 12 kb (Fig. 1). By comparing the fragments detected by the Tn*10* probe with the restriction map of the *E. coli* chromosome derived from the Kohara library, we concluded that *mutR* was contained in clone 329.

Digestion of clone 329 with *Eco*RI releases five fragments of *E. coli* DNA of approximately 0.9, 1.2, 2, 5, and 6 kb. Since mini-Tn*10* has an internal *Eco*RI site, digestion of DB100 DNA with *Eco*RI generates novel restriction fragments not present in DNA from strain DB1, a strain isogenic to DB100 but lacking the mini-Tn*10* insert in *mutR*. The five *Eco*RI fragments of clone 329 were gel purified, radiolabeled with ^{32}P by the random oligo method (3), and hybridized to *Eco*RI-digested DB100 and DB1 DNA. Only the 5-kb *Eco*RI fragment detected *Eco*RI fragments in DB100 DNA not present in DB1 DNA, indicating that this fragment contained at least part of the wild-type *mutR* locus (Fig. 1).

The 5-kb fragment from Kohara clone 329 was subcloned into the *Eco*RI site of pBR329 (1), and the resulting plasmid was transformed into strains containing missense and mini-Tn*10* inserts in *mutR* and then assayed for its ability to complement the *mutR* mutations by measuring the frequency of spontaneous deletions (10). Table 1 shows that pBR329 with the 5-kb insert does complement *mutR* mutations, whereas pBR329 without the insert does not.

Strategy to sequence *mutR*. To determine the sequence of *mutR*, we employed a method developed in this laboratory

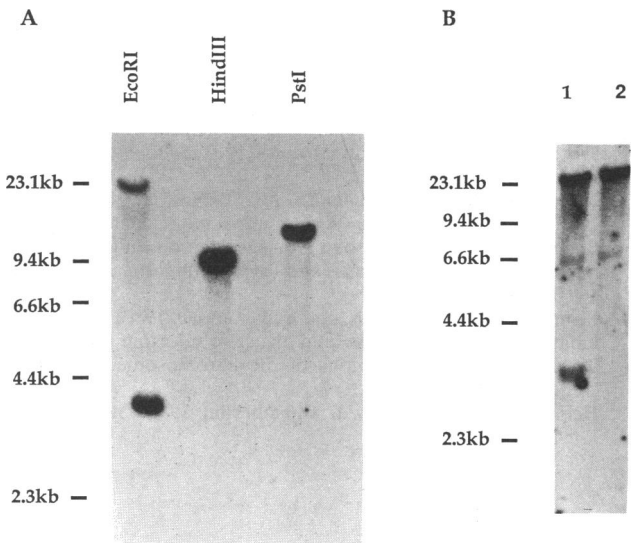

FIG. 1. Southern blots of DB1 and DB100 DNA. (A) DB100 DNA, digested with *Eco*RI, *Hin*dIII, or *Pst*I and hybridized to a radiolabeled oligonucleotide homologous to the ends of Tn*10*. The sizes of the fragments detected (>23- and 3.5-kb *Eco*RI fragments, 9-kb *Hin*dIII fragment, and 12-kb *Pst*I fragment) indicate the *mutR* locus is contained within the Kohara λ clone 329. (B) DB100 (lane 1) and DB1 (lane 2) DNA was digested with *Eco*RI and hybridized to the five *Eco*RI *E. coli*-specific fragments of λ clone 329. Only the 5-kb λ fragment detects fragments of different size in the DB1 and DB100 DNA, indicating the 5-kb fragment of clone 329 contains at least part of the *mutR* locus.

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TABLE 1. Lac$^+$ revertant frequency resulting from deletions

Strain and background[a]	Plasmid	Lac$^+$ revertant frequency (10^8)[b]
DB1, +		58 ± 14
DB1, *topB*		200 ± 24
DB1, *topB*	pBR329	570 ± 170
DB1, *topB*	pBR329 with 5-kb insert	50 ± 5

[a] The strain background was either DB1 (+) or DB100 (*topB*).
[b] The Lac$^+$ revertants result from a deletion of 759 bp at a short homology of 17 bp (10). The values are averages from five or more independent cultures.

(5) for retrieving chromosomal genes disrupted by a mini-Tn*10* element, which is outlined in Fig. 2. Briefly, DB100 was infected with M13mp11*tet*-1, which contains a chloramphenicol marker and an internal fragment of the *tet* gene conferring Tet^r. Since M13mp11*tet*-1 also contains amber mutations in genes I and II, two genes essential for phage

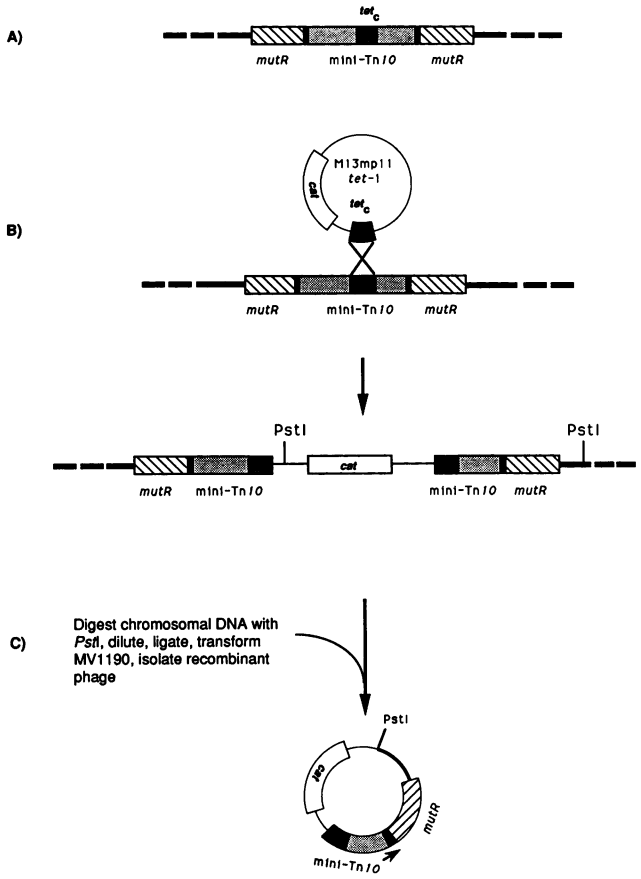

FIG. 2. Strategy to generate DB100 and obtain sequence of the mini-Tn*10* insertion site. (A) DB1 was infected with λ1098 (9) containing the mini-Tn*10* element, to generate DB100 (10). (B) DB100 was infected with M13mp11*tet*-1 to generate MA-1, which is chloramphenicol resistant and tetracycline sensitive. (C) The DNA of MA-1 was digested with *Pst*I, diluted, ligated, and transformed into the *supE*-containing strain, MV1190, to propagate phage-containing *E. coli* sequences. Single-stranded DNA was isolated from the recombinant phage and sequenced with a Tn*10*-specific oligonucleotide primer to obtain the sequence of the adjacent chromosomal DNA. ——, M13 DNA; ▬, chromosomal DNA; →, Tn*10*-specific sequencing primer.

FIG. 3. Sequence analysis. (A) A comparison of 300 nucleotides of *mutR* with 300 nucleotides of *topB* indicate the two loci are identical. (B) The DNA sequence of the junction of mini-Tn*10* and *mutR* indicates the element inserted into the center of the gene at nucleotide position 1279. ↓, mini-Tn*10* insertion site.

replication, selection for Cam^r yields colonies in Su$^-$ strains, such as DB100, only if the M13mp11*tet*-1 has integrated into the chromosome. The integration occurs via homologous recombination between the *tet* gene in DB100 and the *tet* fragment in M13mp11*tet*-1. Integration disrupts the *tet* gene, leading to an M13 lysogen that is Tet^s and Cam^r.

The chromosomal DNA of the Tet^s Cam^r lysogen derived by the integration of M13mp11*tet*-1 into the DB100 chromosome was isolated as described previously (6), digested with *Pst*I, and then ligated with T4 ligase. The ligated DNA was transformed into competent MV1190 (Bio-Rad Laboratories), an Su$^+$ strain, to propagate the recombinant M13 phage. Single-stranded DNA was isolated from the phage, and the sequence of the chromosomal DNA adjacent to mini-Tn*10* was determined by using an oligonucleotide primer complementary to the ends of Tn*10*. This sequence was used subsequently to generate additional primers to sequence the wild-type *mutR* locus subcloned into pBR329 (see above).

Sequence analysis of *mutR*. Our analysis of 1,286 bp of *mutR* sequence indicated that this gene is identical to the *topB* gene encoding topoisomerase III in *E. coli* (2). Topoisomerase III also shares extensive homology to topoisomerase I in *Saccharomyces cerevisiae* (8). Figure 3 shows some of the stretches of sequence for both *mutR* and *topB*.

Role of topoisomerase III in deletion formation. There are several ways in which cells lacking topoisomerase III might have higher deletion rates. Since one activity of topoisomerase III is to relax supercoils, it is conceivable that if the primary pathway for the deletions we are observing is via recombination, then supercoiled intermediates might play a key role. Although the general level of supercoils in the cell is apparently not greatly affected in *topB* strains, it is

possible that specific structures are particularly sensitive to topoisomerase III action. In this case, in the absence of topoisomerase III activity, an increase in the supercoiled structures could lead to an increase in deletion formation. A unimolecular recombination event would loop out a circle, in rough analogy to lambda excision, a reaction which is enhanced by the presence of supercoiled DNA. Interestingly, mutants in *S. cerevisiae* lacking topoisomerase I, which is strongly homologous to topoisomerase III from *E. coli*, display a hyperrecombination phenotype (8). On the other hand, the primary activity of topoisomerase III appears to be decatenation. Future experiments will have to be done to determine the precise intermediates in spontaneous deletion formation.

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