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Specificity of *Escherichia coli mutD* and *mutL* mutator strains

(Recombinant DNA; proofreading; mismatch repair; transition and transversion mutations; hot spots; plasmid pBR322; phage P22)

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SUMMARY

The products of the *mutD* and *mutL* genes of *Escherichia coli* are involved in proofreading by DNA polymerase III and DNA adenine MTase (Dam)-dependent mismatch repair, respectively. We have used the plasmid-borne bacteriophage P22 *mnt* gene as a target to determine the types of mutations produced in *mutL*25 and *mutD*5 strains. Of 60 mutations identified from *mutL*25 cells, 52 were transition mutations and of these the AT → GC subset predominated (40 out of 52). The majority of AT → GC mutations were found at the same three sites (hotspots). In contrast, transversion mutations (47 out of 76) were found about twice as frequently as transitions (28 out of 76) from *mutD*5 bacteria. Two hotspots were identified but at different sites than those in the *mutL*25 cells. These results suggest that the proofreading function of DNA polymerase III primarily repairs potential transversion mutations while Dam-dependent mismatch repair rectifies potential transition mutations.

INTRODUCTION

There are at least two mechanisms by which mismatched bp are corrected in newly synthesized DNA: proofreading and post-replicative mismatch repair. Proofreading is intimately associated with replication and errors are removed as soon as they arise (Kornberg, 1980; 1988; Kunkel, 1988). The proofreading function is determined by the α and ε sub-units of DNA polymerase III holoenzyme which are specified by the *polC* and *mutD* (*dnaQ*) genes respectively (Scheuermann and Echols, 1984; DiFrancesco et al., 1984; Cox and Horner, 1986; Maki and Kornberg, 1987). Mutant *polC* and *mutD* strains of *Escherichia coli* show an increased spontaneous mutation rate which is consistent with the postulated role of these gene products (Hall and Brammar, 1973; Degnan and Cox, 1974; Sevastopoulos and Glazer, 1977; Horiuchi et al., 1978).

The substrate for mismatch repair is duplex DNA containing the newly synthesized unmethylated daughter strand and a methylated parental strand (Claverys and Lacks, 1986; Radman and Wagner, 1986; Modrich, 1987; Meselson, 1988). Hemimethylated Dam-recognition sites (GATC) are substrates for incision by the MutH protein on the unmethylated strand. The MutS protein recognizes mismatched base pairs and the activities of the MutL, DNA helicase II and single-strand binding proteins are required for maximal repair (Modrich, 1987). Thus the bacteria mutants in the *mutH*, *mutL*, *mutS* and *uvrD* (DNA helicase II) genes show elevated spontaneous mutation rates (Cox, 1976).

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Abbreviations: Ap, ampicillin; *bla*, gene encoding β-lactamase; bp, base pair(s); Dam, DNA adenine MTase; *dam*, gene encoding Dam; *dis*, heteroimmune (dismune); hy, hybrid; *imm*, immunity; IS, insertion sequence; *mnt*, bacteriophage P22 gene involved in maintenance of lysogeny; MTase, methyltransferase; *mut*, gene whose mutant allele results in a mutator phenotype; nt, nucleotide(s); O^c, operator constitutive; *ori*, origin of DNA replication; ^{R}, resistance/resistant; ^{S}, sensitive; Tc, tetracycline; *tet*, gene in pBR322 encoding for Tc^R; *tsp*, transcription start point; wt, wild type.

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The aim of the present studies is to show that the mutation spectrum is the same in mismatch repair-deficient or defective strains but different from that in proofreading-defective bacteria.

A preliminary account of this work was presented at the New England Biolabs Workshop on Biological DNA Modification (Carraway et al., 1988).

MATERIALS AND METHODS

(a) Bacterial strains and plasmids

Strains ES1582 (*mutL*25) and ES1578 (*mutD*5) are derivatives of AB1157 and were obtained from Dr. E. Siegel, Tufts University, Medford, MA. Plasmids F42 (F′*lac*) and pPY97 were introduced into these strains to yield GM3134 and GM3150, respectively. Strain MM294 was used as the host strain from which plasmid DNA was prepared for DNA sequencing and was obtained from the *E. coli* Genetic Stock Center (B. Bachmann, Curator, Dept. of Biology, Yale University, New Haven, CT). Plasmid pPY97 is a derivative of pBR322 containing the *mnt* gene and an M13 *ori* (Lucchesi et al., 1986; Rewinski and Marinus, 1987).

(b) Other methods

Cells were grown in Brain Heart Infusion (BHI) broth at 37°C and bacteria and mutations in *mnt* were isolated as previously described (Lucchesi et al., 1986; Carraway et al., 1987; Rewinski and Marinus, 1987). Briefly, a portion from each of a series of overnight standing BHI broth cultures containing 40 μg Ap/ml was plated on medium containing 3.5 μg Tc/ml and 40 μg Ap/ml. After overnight incubation, colonies were restreaked twice on BHI broth plates containing 10 μg Tc/ml. The Tc^R isolates were lysogenized with phage λimm^{P22}hy1 (Yamamoto et al., 1978) and challenged with phage $\lambda imm^{P22}dis$ (Yamamoto et al., 1978). Lysogens sensitive to superinfection have mutations in the *mnt* gene whereas those which are resistant contain mutations in the Mnt operator region (Lucchesi et al., 1986). Plasmids from Mnt^- cells were transferred into strain MM294 and supercoiled plasmid DNA was isolated and sequenced as described by Rewinski and Marinus (1987).

RESULTS AND DISCUSSION

(a) Experimental system

The *mnt* gene of phage P22 was used as a target to monitor and identify the specificity of mutagenesis. This gene is present on a plasmid (pPY97) which was used to transform each mutator strain. Since the Tc^R gene (*tet*) of pPY97 is under the negative control of the Mnt repressor, forward mutations that inactivate the *mnt* gene or its operator confer a Tc^R phenotype. This selective system has been described in detail elsewhere (Lucchesi et al., 1986; Carraway et al., 1987; Rewinski and Marinus, 1987).

Spontaneous Tc^R cells arise in wt cultures at a frequency of about 1×10^{-8}. For *dam*-deficient strains, the frequency is increased about 15-fold and for *mutH* and *mutL* mutants about 100-fold. The frequency in *mutD* cultures is about 1000-fold above the wt level. In contrast to the wt, where greater than 95% of the Tc^R mutations map to the operator, between 50–90% of the mutations from the mutator strains are in the *mnt* gene.

(b) Distribution and frequency of spontaneous mutations from the *mutL*25 strain

Table I lists 60 independent mutations in the *mnt* gene identified by nt sequencing which arose in the *mutL*25

TABLE I

Analysis of mutations in the *mnt* gene arising in a *mutL*25 strain

Position [a] (nt)	nt change	aa change	Number [b]
-44/-34	Deletion	Promoter	1
-11	A→G	Promoter	1
24	G→A	Met→Ile	2
28	A→G	Arg^2→Gly	1
40	C→T	His^6→Tyr	2
41	A→G	His^6→Arg	16
47	A→G	Asn^8→Ser	1
52	C→T	Arg^{10}→Cys	2
53	G→A	Arg^{10}→His	1
62–63	+C	Frameshift	1
71–73	-G	Frameshift	1
80	T→C	Leu^{19}→Ser	2
84	A→C	Lys^{20}→Asn	1
89	G→T	Arg^{22}→Met	1
89–91	+G	Frameshift	1
92	C→T	Ala^{23}→Val	2
103–105	-G	Frameshift	1
103	G→T	Gly^{27}→Trp	1
106	A→G	Arg^{28}→Gly	7
109	T→C	Ser^{29}→Pro	1
110	C→T	Ser^{29}→Leu	2
115	A→G	Met^{30}→Val	2
116	A→G	Asn^{31}→Ser	4
121	G→A	Glu^{33}→Lys	1
122	A→G	Glu^{33}→Gly	2
149	T→C	Leu^{42}→Pro	1
273	A→G	Stop→Trp	2

[a] Numbered with respect to the *tsp* (+1) of the *mnt* gene. The start codon is at nt 21–23 and the stop codon at nt 271–273. Mutations were isolated and characterized from strain GM3134, a *mutL*25 derivative of AB1157 bearing plasmids F′42 and pPY97, as described in MATERIALS AND METHODS. The same methods were used to isolate the mutations described in Table III except that strain GM3150, a *mutD5* derivative of AB1157, was used.

[b] Number of identical mutants isolated.

TABLE II

Type and frequency of mutations arising in the *mnt* gene in wt and mutator strains

Type of mutation	Allele[a] (frequency of mutations[b])				
	wt	*dam*-3	*mutH*34	*mutL*25	*mutD*5
IS element	10/13	6/90	1/47	0/60	0/76
Deletion	3/13	2/90	1/47	1/60	1/76
Frameshift	0/13	5/90	0/47	4/60	0/76
Transition	0/13	73/90	45/47	52/60	28/76
AT → GC		62/73	32/45	40/52	12/28
GC → AT		11/73	13/45	12/52	16/28
Transversion	0/13	4/90	0/47	3/60	47/76
AT → TA		1/4		0/3	13/47
AT → CG		2/4		1/3	27/47
GC → CG		0/4		0/3	7/47
GC → TA		1/4		2/3	0/47

[a] The data for the *dam*, *mutH* and wt strains are taken from Carraway et al. (1987) and Rewinski and Marinus (1987). The data for the other strains are from this paper (Tables I and III). See footnotes to Table I.
[b] The number of each type of mutation as a fraction of the total.

background. Table II summarizes the data for this strain as well as the wt and other mutator bacteria. In the *mutL*25 cells, transition mutations comprised the major class (52/60) and of these the AT → GC subset predominated (40/52). The other eight identified mutations were a deletion, four frameshifts and three transversions. The deletion included nt −34 to −44 which eliminated the −35 region of the *mnt* promoter. Three of the frameshifts involved insertion or deletion of a G residue in a run of three consecutive G (at nt 71–73, 89–91 and 103–105). Such frameshifts are not unusual and are hypothesized to arise by 'slippage' of DNA strands (Streisinger et al., 1966). The fourth frameshift is unusual in that it is an insertion of a C residue between T^{62} and G^{63}.

(c) Comparison of mutation spectra in strains defective in mismatch repair

Table II compares the mutation spectra in *dam*-3 (Carraway et al., 1987), *mutH*34 (Rewinski and Marinus, 1987) and *mutL*25 strains. They are strikingly similar to each other, but are different to those of the wt and *mutD*5 strains. In particular, the strains defective in mismatch repair yielded primarily transition mutations and, at a lower frequency, transversions and frameshifts. In these respects, the results with *mnt* are in agreement with those obtained in the *lacI* system (Leong et al., 1986; Schaaper and Dunn, 1987). The combined data argue that the products of all these genes are involved in the rectification of nt mispairs by Dam-dependent mismatch repair.

The transition mutations can arise from either A · C or G · T mispairs. It is not possible to determine from the data in Tables I and II which of these, or both, is responsible for the mutations we have detected. More recent data obtained by in vitro experiments indicate that both mispairs are formed for *mnt* (M. Carraway, C. Rewinski and M.G.M., unpublished data).

The AT → GC class of mutations in *mnt*, however, is three to six times more frequent than the GC → AT transition class. This bias is due to the frequent occurrence of AT → GC transitions at the same three sites (hotspots), i.e., at nt positions 41, 106 and 116. Fig. 1 shows that all three mismatch-repair-defective strains (*dam*-3, *mutH*34 and *mutL*25) yield these hotspots. The hotspot sites are within six bp of the only two Dam-recognition (GATC) sequences in the *mnt* gene, at nt positions 34–37 and 107–110.

Table II shows that no transition or transversion mutations were detected in the wt strain. Systems which repair mismatches, therefore, must be very efficient in the wt background.

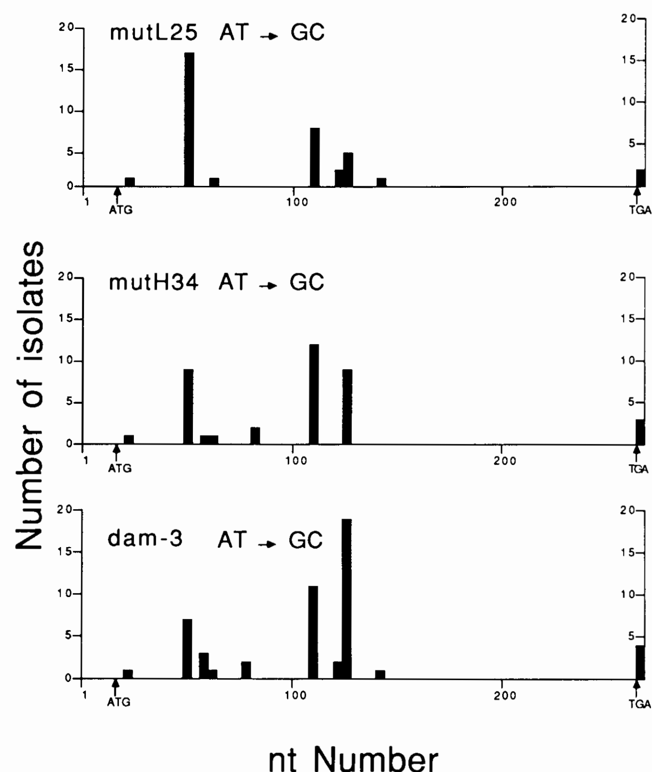

Fig. 1. Location of AT → GC mutational hotspots in the *mnt* gene obtained from the indicated bacterial strains. The number of AT → GC transitions at selected nucleotides in the *mnt* gene from *mutL*25 (Table I), *dam*-3 and *mutH*34 strains (Carraway et al., 1987; Rewinski and Marinus (1987) is displayed. The three hotspots at nt positions 41, 106 and 116 are present in all these strains. They were not prominent in either the *mutD*5 (Table III) or the wt strains (Carraway et al., 1987; Rewinsky and Marinus, 1987). Numbering of nt begins at the *tsp* (Lucchesi et al., 1986). The mutations at nt 273 are in the stop codon.

(d) Distribution and frequency of spontaneous mutations from the *mutD5* strain

Table III lists 76 mutations in *mnt* which were recovered from *mutD*5 cells. These represent 60% of the total Tc^R isolates; the remainder were phenotypically O^C. In contrast to the other mutator strains (Table II), transversion mutations were about twice as frequent (47/76) as transitions (28/76). Apart from the two hotspot sites described below, the mutations were uniformly distributed throughout the proximal part of the *mnt* gene and in the translational termination codon. Table III also shows that a deletion mutation was recovered which eliminated bp 194–199. This was the only deletion mutation greater than one bp that we have ever recovered in the *mnt* coding region.

The distribution of transition mutations from *mutD*5 bacteria was distinctly different from those of the other mutator strains (Table II). The hotspots characteristic of mismatch-repair-defective strains at nt 41, 106 and 116 were absent and a new hotspot site was located at nt −33 in the promoter region (Table III). Also in contrast to the mutator strains described in Table II, the number of AT → GC changes is approximately equal to the GC → AT class in *mutD*5 cells.

The majority of the transversions occurred at A · T bp (40 out of 47) and of these 25 changed T^{109} to A or G (Table III). This T residue is within the Dam-recognition site at nt 107–110. Since no hotspot was detected at the other GATC in *mnt*, the mechanism of hotspot formation must be independent of Dam-recognition sites.

The data in Table III indicate that under the experimental conditions we have used to isolate mutations in *mnt* there is little, if any, inhibition of Dam-dependent repair in *mutD*5 bacteria since the spectrum of transition mutations does not resemble that for mismatch repair defective strains (cf., Schaaper, 1988; Isbell and Fowler, 1989). Consequently, it is reasonable to conclude that in the absence of proper proofreading by DNA polymerase III in *mutD*5 cells both transition and transversion mutations are produced in *mnt* with the latter comprising the major class.

TABLE III

Mutations in the *mnt* gene arising in a *mutD*5 strain

Position [a]	nt change	aa change	Number [b]
−36	A → C	Promoter	3
−35	T → C	Promoter	3
−33	G → A	Promoter	9
24	G → A	fMet → Ile	1
26	C → T	Ala^1 → Val	1
28	A → G	Arg^2 → Gly	1
40	C → T	His^6 → Tyr	2
41	A → T	His^6 → Leu	2
41	A → G	His^6 → Arg	1
42	C → G	His^6 → Gln	3
46	A → T	Asn^8 → Ile	1
53	G → A	Arg^{10} → His	1
55	A → T	Met^{11} → Leu	1
70	A → T	Arg^{16} → Trp	3
77	A → C	Lys^{18} → Thr	2
91	G → C	Ala^{23} → Pro	3
103	G → A	Gly^{27} → Arg	1
106	A → G	Arg^{28} → Gly	1
108	A → T	Arg^{28} → Stop	1
109	T → G	Ser^{29} → Ala	20
109	T → A	Ser^{29} → Thr	5
110	C → T	Ser^{29} → Leu	1
116	A → G	Asn^{31} → Ser	2
137	T → G	Val^{38} → Gly	1
176	A → C	Tyr^{51} → Ser	1
194–199	Deletion		1
199	G → C	Ala^{59} → Pro	1
273	A → G	Stop → Trp	4

[a,b] See footnotes a, b to Table I.

(e) Intergenic deletions were not included in the analysis

For each of the mutator strains listed in Table II, about 1–10% of the Tc^R isolates were Ap^S. This is due to deletion mutations which have one end at variable sites within *mnt* and the other end at variable points in the gene (*bla*) encoding β-lactamase. We have not included these deletions in the analysis because this kind of deletion was found in pBR322 derivatives without the *mnt* gene (Yi et al., 1988; S. Bouvier and M.G.M., unpublished data). We believe, therefore, that these deletions are a reflection of the plasmid nature of the target and may not be representative of events occurring on the host chromosome.

(f) An unexpected feature

An unexpected feature of the spectra in Tables I and III, is that mutations are found almost exclusively in the DNA corresponding to the N-terminal half of the protein. At present, it is unclear why this should be. The Mnt repressor does not display the helix-turn-helix motif found in many DNA-binding proteins (Knight et al., 1989) and residues in both N-terminal (Knight and Sauer, 1989) and C-terminal (Knight and Sauer, 1988) parts of the repressor are necessary for DNA binding. Mnt repressor binds to DNA as a tetramer (Vershon et al., 1985) and to both the 'front' and 'back' of the operator (Vershon et al., 1987).

Given these properties one might expect that frameshift or nonsense mutations affecting the C-terminal end of the repressor should be recovered in vivo from the mutator strains. That they have not been found in vivo is puzzling, especially since nonsense mutations affecting the C-terminal residues which perturb DNA binding have been isolated by site-directed mutagenesis (Knight and Sauer, 1988).

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