Escherichia coli Mutator mutD5 Is Defective in the mutHLS Pathway of DNA Mismatch Repair

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ABSTRACT

We have previously reported that the Escherichia coli mutator strain mutD5 was defective in the correction of bacteriophage M13mp2 heteroduplex DNA containing a T-G mismatch. Here, this defect was further investigated with regard to its interaction with the mutHLS pathway of mismatch repair. A set of 15 different M13mp2 heteroduplexes was used to measure the mismatch-repair capability of wild-type, mutL and mutD5 cells. Throughout the series, the mutD5 strain proved as deficient in mismatch repair as the mutL strain, indicating that the repair defect is similar in the two strains in both extent and specificity. [One exception was noted in the case of a T-G mispair that was subject to VSP (Very Short Patch) repair. VSP repair was abolished by mutL but not by mutD.] Variation in the dam-methylation state of the heteroduplex molecules clearly affected repair in the wild-type strain but had no effect on either the mutD or mutL strain. Finally, mutDmutL or mutDmutS double-mutator strains were no more deficient in mismatch repair as were the single mutator strains. The combined results strongly argue that the mismatch-repair deficiency of mutD5 cells resides in the mutHLS-dependent pathway of mismatch repair and that the high mutation rate of mutD strains derives in part from this defect.

Escherichia coli mutD mutator strains are very strong mutators, their mutation rates often being elevated 10,000–100,000-fold above the wild-type levels (Degen and Cox 1974; Cox and Horner 1982). The mechanism(s) responsible for these high rates are of interest for understanding the pathways that control mutation. The primary defect of mutD strains resides in the dnaQ gene (Schaeermann et al. 1983; Maruyama et al. 1983; Schaeermann and Echols 1984; DiFrancesco et al. 1984). The dnaQ gene encodes the proofreading exonuclease (ε-subunit) of the DNA polymerase III (Pol III) holoenzyme which is responsible for the faithful duplication of the bacterial chromosome. Two types of mutators have been described at this locus: mutD mutators which are dominant over wild type and whose phenotype is conditional on the growth medium (strong mutator in rich medium but only moderate in minimal medium) (Cox 1976; Cox and Horner 1982), and dnaQ mutators which are recessive and temperature-sensitive for both mutator phenotype and DNA synthesis (Horwich, Maki and Sekiguchi 1978; Maki, Horwich and Sekiguchi 1983). In either case, the cell’s proofreading ability is believed to be compromised, causing faulty (i.e., inaccurate) DNA synthesis. A reduction in exonuclease has, indeed, been demonstrated with Pol III preparations from both mutD5 and dnaQ49 strains (Echols, Lu and Burgers 1983). A defective but tightly binding ε-subunit in mutD mutators and a weak or nonbinding subunit in dnaQ mutators was suggested to account for their respective dominant or recessive characters (Cox and Horner 1986; Takano et al. 1986). The conditional (i.e., medium-dependent) nature of the mutD mutators is as yet unexplained.

Our interest in mutD arose from a desire to use this strain to determine the efficiency and specificity of in vivo proofreading. However, a straightforward interpretation of mutD mutational data in terms of a proofreading defect is suspect in view of the large magnitude (10⁻⁴ to 10⁻⁵-fold) of the mutator effect. The fidelity of DNA Pol III holoenzyme is estimated to be (2–5) × 10⁻⁷ (for the more frequent mispairs) (Fersht and Knill-Jones 1983), which is achieved by the combined contributions of insertion fidelity and proofreading. The insertion fidelity has not yet been measured but may be reasonably estimated at 10⁻⁴ to 10⁻⁵ (Loeb and Kunsel 1982). If this value is correct, the contribution from proofreading should be smaller (20–500-fold) than indicated by the mutD mutator effect. Furthermore, Fersht, Knill-Jones and Tsui (1982) estimated the maximum contribution of proofreading by Pol III holoenzyme at about 200-fold (although this estimate depended on the (untested) equation of the insertion fidelities of DNA polymerases I and III). The cost of proofreading (in the form of increased hydrolysis of correct nucleotides) was very high and seemed to preclude a much larger contribution of proofreading.
The specificity of mutagenesis in mutD cells in minimal and rich media (SCHAAPER 1988) is markedly different: in minimal medium transversions are most frequent, in rich medium, transitions. On the basis of this disparity, I proposed that mutD might contain two different defects which may be differentially expressed depending on the growth conditions. The first is the proofreading defect, which may be largely responsible for the mutator effect in minimal medium. The second defect, which is an additional defect and is expressed only in rich medium, was proposed to be a deficiency in postreplicative DNA mismatch repair.

Postreplicative mismatch repair in E. coli is encoded by the mutH, mutL, and mutS genes (for recent reviews see CLAVERYS and LACKS 1986; RADMAN and WAGNER 1986; and MODRICH 1987). The mutHLS system acts shortly after DNA synthesis, recognizing and correcting mismatches that result from DNA replication errors using the transient undermethylation (at GA/TC sites) of the newly replicated strand to distinguish the "correct" from the "incorrect" half of the mismatch. The overall contribution of this system to lowering mutation rates is about 300-fold (SCHAAPER and DUNN 1987a), although it is far more efficient at correcting transitions than transversions (KRAMER, KRAMER and FRITZ 1984; CHOW and FOWLER 1985; DOHET, WAGNER and RADMAN 1985; SU et al. 1988). The hypothesis that mutD cells may be conditionally defective in mismatch repair is consistent with this intrinsic specificity: in minimal medium, transitions are strongly suppressed by the action of mismatch repair, causing an apparent excess of transversions; however, abolition of mismatch repair in rich medium leads to an increase in the mutation frequency and a shift of the mutational spectrum in favor of the transitions.

Proof of the proposed mismatch-repair deficiency requires direct measurements of the capacity of mutD cells to perform mutHLS-dependent mismatch correction. Indeed, competent cells derived from a mutD5 strain proved deficient in the correction of a transfected heteroduplex M13mp2 DNA containing a T-G mismatch (SCHAAPER 1988). In this study, we have evaluated the ability of mutD5 cells to correct a large number of different mismatches at several nucleotide positions and have compared the results with those of wild-type and mutL cells. We have also investigated the effects of dam methylation and the extent of mismatch correction in strains doubly deficient in the mutD and mutL or mutS genes. The results provide strong support for the hypothesis that mutD cells are deficient in mutHLS-dependent mismatch repair and that a large fraction of the mutD mutator effect results from this deficiency.

## MATERIALS AND METHODS

### Strains:

The bacterial and phage strains used in this study are described in Table 1.

### M13 heteroduplex DNAs:

M13mp2 heteroduplex DNAs containing a nick in the complementary strand (RF I II DNAs) were obtained as follows. Single-stranded DNA of phage M13mp2 (amA88) or M13mp2 (ocT90) was prepared from M13 virions grown on host CSH50 (dam*) or NR3745 (dam*) by the method of BIRNBAUM (1983), followed by CsCl/ethidium-bromide ultracentrifugation to obtain essentially pure (>90%) supercoiled RF I DNA. The dam* or dam- state of the RF I DNA was confirmed by restriction analysis with the enzymes DpnI (cutting only methylated DNA), MboI (cutting only unmethylated DNA), and Sau3A1 (cutting regardless of methylation). The RF I DNAs were then digested to completion with the restriction enzyme AvaII, which cuts M13mp2 only once at nucleotide +264 (position 1 being the first transcribed base of the lacZa gene in M13mp2), followed by removal of the restriction enzyme by phenol extraction, ethanol precipitation and resuspension of the linearized (RF I) DNA in TE (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). Heteroduplex DNAs were then prepared by a modification of the method of KUNKEL and SONI (1988). A 25-μl aliquot of RF III DNA (5-10 μg) was diluted with 375 μl H2O and denatured by heating at 70° for 10 min, after which 7 μl (25 μg) single-stranded DNA (amA88 or ocT90) were added. After 2 min of further incubation at 70°, 100 μl 10× SSC were added and the mixture transferred to 60° for 10 min (hybridization step). Agarose-gel electrophoresis was used to confirm the disappearance of the linear, double-stranded RF III DNA and the appearance of circular (heteroduplex) RF II DNA (>90% conversion). The excess of single-stranded DNA was removed by extraction with benzoylated naphthoylated DEAE-cellulose in 1 M NaCl (GAMPER et al. 1985) followed by ethanol precipitation and resuspension in TE. The resulting DNA is essentially (>90%) heteroduplex DNA, the remainder being a small amount of homoduplex linear DNA. Upon transfection of this DNA preparation into competent cells (see below), the only biological activity (i.e., plaque formation) results from the heteroduplex RF II DNA.

### Transfections and measurement of mismatch repair:

Competent cells (HANAHAN 1983) were prepared freshly for each experiment and used, as a rule, within 1 hr. A 0.5-ml aliquot of competent cells was incubated with ~10 ng of heteroduplex DNA at 0° for 20 min, briefly heat-shocked (90 sec at 42°) and returned to ice. Multiple 2-, 10- and 50-μl aliquots of the transfection mixture were then plated on minimal medium in soft-agar layers (SCHAAPER and DUNN 1987b) containing indicator strain CSH50, 0.5 mg isopropyl-β-D-thiogalactopyranoside (IPTG), and 1.0 mg 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). After incubating for 16–24 hr at 37°, the resulting plaques were assigned to one of the following categories: pure blue, pure white, or mixed blue/white. This analysis, when performed on plates containing about 300 or fewer plaques, can be done with confidence (>95% accuracy) as judged from control experiments in which plaques were first assigned, then picked and individually replated.

## RESULTS

### Measurement of DNA mismatch repair:

To assess the ability of E. coli mutD5 strains to perform mismatch correction, we constructed a series of bacteriophage M13mp2 RF II DNA molecules, each containing a defined single-base-pair mismatch (Figure 1).
M13mp2 contains the E. coli lacZα gene and is capable of α-complementation (blue plaque formation on host CSH50 in the presence of X-gal and IPTG) (MESSING et al. 1977). The α-complementing ability can be abolished by single lacZα point mutations yielding colorless or "white" plaques (KUNDEL 1984). We used two colorless derivatives carrying a TAG (amber) or TAA (ochre) mutation, respectively (Figure 1). Various studies on the specificity of mutagenesis (SCHAAPER and DUNN 1987b, 1988, and unpublished data) provided revertants that had regained the capacity for α-complementation. DNA sequence analysis revealed that all 15 single-base-pair substitutions that restored a sense codon yielded a blue-plaque phenotype. This set of nonsense mutants and their 15 revertants were used to construct 15 different heteroduplex DNA, containing an A-C mismatch, transfected into wild-type, mutL, and mutD5 strains. Two types of heteroduplexes were used, differing in dam methylation. In the first, both strands were de- methylated (or undermethylated) strand is observed, a mixed plaque will be observed containing both white and blue progeny. The distinction between the three types of plaques, easily performed visually, provides a rapid measure of in vivo mismatch repair. This use of M13 (or f1) heteroduplex molecules to study mismatch repair, in vivo as well as in vitro, has been described before (LU, CLARK and MODRICH 1983; KRAMER, KRAMER and FRITZ 1984; SHENOY, EHRLICH and EHRLICH 1987; SU et al. 1988).

**Mismatch repair by mutD5 and effect of methylation:** Table 2 shows the results of experiments using heteroduplex DNA, containing an A-C mismatch, transfected into wild-type, mutL, and mutD5 strains. Two types of heteroduplexes were used, differing in dam methylation. In the first, both strands were derived from a dam+ host (me+/me+ DNA). In the second, the complementary strand was obtained from a dam- strain, producing a hemi-methylated molecule (me+/me- DNA). Both heteroduplexes are efficiently repaired when transfected into a wild-type host, only ~4% of the infective centers producing mixed plaques (Table 2). That this represents mutHLS-dependent mismatch repair is indicated by the strong increase in the number of mixed bursts in the mutL strain (43.0-52.5% mixed) and the marked effect of methylation on the directionality of repair. The me+/me- molecules are corrected preferentially towards the blue phenotype (viral strand repair), me+/me- molecules towards the white phenotype (complementary strand repair). M13 viral DNA obtained from a dam+ strain is inefficiently methylated (Lu et al. 1984). Therefore, in both cases, preferential correction of the unmethylated (or undermethylated) strand is observed, a characteristic feature of mutHLS-dependent mismatch repair.

Competent cells derived from the mutD5 strain are severely affected in their ability to perform mismatch repair, yielding a percentage of mixed bursts close to that of the mutL strain (Table 2). The reduction in mismatch repair may be calculated to be 84% ([36.5-3.4]/[43.0-3.4]) and 87% ([46.3-4.6]/[52.5-4.6]) for the A-C heteroduplex in the two methylation states, respectively. The residual amount of mismatch

### Table 1

**Bacterial and phage strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Origin/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSH50</td>
<td>ara, thi, Δprolac, strA, F'prolac*ZΔM15</td>
<td>KUNDEL (1984)</td>
</tr>
<tr>
<td>KA796</td>
<td>ara, thi, Δprolac, F'</td>
<td>P90C (MILLER et al. 1977)</td>
</tr>
<tr>
<td>NK3745</td>
<td>ara, thi, dam+Δprolac, F'prolac</td>
<td>GICKL (1979)</td>
</tr>
<tr>
<td>NK830</td>
<td>ara, thi, Δprolac, mutH101</td>
<td>F' of NR3939 (SCHAAPER and DUNN 1987a)</td>
</tr>
<tr>
<td>NR880</td>
<td>ara, thi, Δprolac, mutD101</td>
<td>F' of NR3940 (SCHAAPER and DUNN 1987a)</td>
</tr>
<tr>
<td>NR8906</td>
<td>ara, thi, Δprolac, trpE9777, saf-13::Tn10mutD5</td>
<td>F' of NR3966 (SCHAAPER and DUNN 1987a)</td>
</tr>
<tr>
<td>NR9163</td>
<td>KA796, but mutL218::Tn10</td>
<td>FOWLER, SCHAAPER and GICKL (1986)</td>
</tr>
<tr>
<td>NR9164</td>
<td>KA796, but mutS215::Tn10</td>
<td>P1(KD1094) (R. FOWLER) (tet' selection)</td>
</tr>
<tr>
<td>NR9174</td>
<td>NR8040, but saf-13::Tn10mutD5</td>
<td>P1(KD1094) (R. FOWLER) (tet' selection)</td>
</tr>
<tr>
<td>NR9184</td>
<td>NR8040, but saf-13::Tn10mutD5</td>
<td>P1(KD1094) (tet' selection)</td>
</tr>
<tr>
<td>NR9194</td>
<td>NR8041, but saf-13::Tn10mutD5</td>
<td>P1(KD1094) (tet' selection)</td>
</tr>
</tbody>
</table>

**Bacteriophages**

| M13mp2   | lacZα*            | MESSING et al. (1977) |
| M13mp2(A88) | lacZα* (amber)   | KUNEL (1984)        |
| M13mp2(T90) | lacZα* (ochre)  | KUNEL (1984)        |

**Materials and Methods**: The resulting mismatches are indicated within parentheses in Figure 1 (line III). Line IV provides the example of an A-C mispair (position 88). Transfection of the heteroduplex into competent E. coli cells and analysis of the resulting infective centers reveals the fate of the mispair. If repair of the mismatch occurs prior to replication, a blue plaque will result (either white or blue, depending on the strand repaired). If no repair occurs, a mixed plaque will be observed containing both white and blue progeny. The distinction between the three types of plaques, easily performed visually, provides a rapid measure of in vivo mismatch repair. This use of M13 (or f1) heteroduplex molecules to study mismatch repair, in vivo as well as in vitro, has been described before (LU, CLARK and MODRICH 1983;
FIGURE 1.—Transfection of M13mp2 heteroduplex DNA to measure DNA mismatch repair. I, DNA sequence of lacZα (codons 15 through 18) contained in phage M13mp2. II, Single-base substitutions at nucleotides 88 or 90 yield a nonsense codon that abolishes the α-complementing ability of the phage (white plaque). III, From these nonsense mutants, revertants are obtained that have regained the ability to α-complement (blue plaques). These revertants include all 15 possible conversions (by a single-base change) to a sense codon (R. M. Schaaper, unpublished data). Heteroduplexes can be constructed by combining DNA from each revertant with that of its nonsense parent. By annealing the viral circle of a nonsense phage to the complementary strand of the revertants, 15 different heteroduplexes can be created containing the mismatch indicated within parentheses (viral or complementary). IV, The example of an A-C heteroduplex (mismatch at position 88: TAG amber combined with TGG revertant). (v) indicates viral or (+) strand, (c) indicates complementary or (−) strand. The complementary strand contains a nick as a result of the construction procedure (see MATERIALS AND METHODS). The viral strand was in all cases obtained from a dam+ host, the complementary strand from either a dam+ or dam− host. Transfection of the heteroduplex DNA in competent cells and immediate plating to obtain infective centers allows the assessment of mismatch repair in the chosen host.

TABLE 2
Mismatch repair of M13mp2 heteroduplex (A-C)α by wild-type, mutL, and mutD5 strains

<table>
<thead>
<tr>
<th>Methylation (v/c)</th>
<th>Strain</th>
<th>Genotype</th>
<th>Mixed</th>
<th>White</th>
<th>Blue</th>
<th>No. of expts.</th>
</tr>
</thead>
<tbody>
<tr>
<td>me+/me+</td>
<td>KA796</td>
<td>mut1</td>
<td>3.4</td>
<td>22.8</td>
<td>73.8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>NR9163</td>
<td>mutL</td>
<td>45.0</td>
<td>14.9</td>
<td>42.1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>NR9066</td>
<td>mutD</td>
<td>36.5</td>
<td>13.4</td>
<td>50.1</td>
<td>6</td>
</tr>
<tr>
<td>me+/me−</td>
<td>KA796</td>
<td>mut1</td>
<td>4.6</td>
<td>75.8</td>
<td>19.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>NR9163</td>
<td>mutL</td>
<td>52.5</td>
<td>9.3</td>
<td>38.2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>NR9066</td>
<td>mutD</td>
<td>46.3</td>
<td>20.5</td>
<td>33.1</td>
<td>7</td>
</tr>
</tbody>
</table>

* dam methylation of viral (v) and complementary (c) strands.

* Average of three to seven determinations. The total number of plaques per determination was 1000 or more.

repair in the mutD strain (comparing mutD to mutL) produces mostly blue plaques from the me+/me+ heteroduplex, but mostly white plaques from the me+/me− heteroduplex, as was the case in the wild-type strain. While the heteroduplex transfection assay yields around 50% pure bursts, even in a mutL strain (leaving room for the possible involvement of other mismatch-repair systems), the data are highly consistent with mutD strains being deficient in mutHLS-dependent repair.

The specificity of the mutD-associated mismatch-repair defect: mutHLS-dependent mismatch repair has a characteristic specificity: transition mismatches (T-G and A-C) are well repaired, transversion mismatches (purine-purine and pyrimidine-pyrimidine pairs) are poorly repaired (although efficiencies are
Mismatch Repair in *E. coli* mutD5

**Figure 2.** Mismatch repair in *E. coli* mut*, mutL*, and mutD5 strains for eight different mismatches at a TAG amber codon (amA88). The percentage of mixed bursts (infective centers) is an inverse indicator of mismatch repair: a low percentage indicates efficient repair, a high percentage little or no repair. See Figure 1 for sources and locations of the mismatches. □, KA796 (wild type); ○, NR9163 (mutL::TnlO); ▲, NR9066 (mutD5). Each point represents the average value for 2–7 determinations. The total number of infective centers screened for each determination averaged 1000 (range 500–4000). The data for the pure plaques are not presented here, but were essentially as in Table 2 (me+/me-), i.e., when repair occurred, an excess of white plaques was observed and when no repair occurred (e.g., the mutL, data points), blue plaques were more frequent than white. The latter bias may be due to differential strand expression during phage replication.

**Figure 3.** Mismatch repair in *E. coli* mut*, mutL*, and mutD5 strains for seven different mismatches at a TAA ochre codon (ocT90). □, KA796 (wild type); ○, NR9163 (mutL::Tn10); ▲, NR9066 (mutD5). See Legend to Figure 2 for further details.

variable and depend on the neighboring DNA sequence (Krämmer, Kramer, and Fritz 1984; Dohet, Wagner, and Radman 1985; Jones, Wagner, and Radman 1987a; St et al. 1988). Figures 2 and 3 show the results for all 15 mismatches (me+/me- configuration). The percentage of mixed bursts is presented as the most convenient indicator for the efficiency of repair. In wild-type cells, the three transition mismatches (TG87, AC88, and TG90) are well repaired. Of the 12 transversion mismatches, several (GG89, GA89, TC90, AM92, and AG92) are also well repaired. The others (TT87, TC87, AA88, AG88, TT90, and AG90) are poorly repaired, if at all. As expected, the mutL strain is generally deficient in mismatch repair, yielding between 50% and 60% mixed bursts regardless of the mismatch. With only one exception (see below), the mutD5 strain is as deficient as the mutL strain, mimicking mutL not only in the percentages of mixed bursts, but also in the ratio of white to blue plaques (data not shown, but very similar to the A-C-mismatch data of Table 2). The data are entirely consistent with the notion that the mutD-associated mismatch-repair deficiency resides in the mutHLS pathway of mismatch repair.

**mutD and VSP (Very Short Patch) repair.** The exception noted above to the identical behavior of mutD and mutL cells is the T-G mismatch at position 87: this mismatch is not repaired in the mutL strain but is significantly repaired in the mutD strain. The complete data for this mismatch are presented in Table 3 for me+/me+ and me+/me- configurations. The viral-strand sequence for this mismatch reads CTAGG for me+/me+. The data are entirely consistent with the notion that the mutD-associated mismatch-repair deficiency resides in the mutHLS pathway of mismatch repair.

**Table 3.** Mismatch repair of M13mp2 heteroduplex containing mispair (T-G)87: Effect of VSP repair

<table>
<thead>
<tr>
<th>Methylation (v/c)*</th>
<th>Strain</th>
<th>Genotype</th>
<th>Plaque phenotype (%)</th>
<th>No. of expts.</th>
</tr>
</thead>
<tbody>
<tr>
<td>me+/me+ KA796</td>
<td>mut*</td>
<td>&lt;0.9</td>
<td>10.0 89.1 4</td>
<td></td>
</tr>
<tr>
<td>NR9163 mutL</td>
<td>37.1</td>
<td>10.2</td>
<td>52.8 4</td>
<td></td>
</tr>
<tr>
<td>NR9066 mutD</td>
<td>7.7</td>
<td>4.9</td>
<td>87.4 6</td>
<td></td>
</tr>
<tr>
<td>me+/me- KA796</td>
<td>mut*</td>
<td>3.3</td>
<td>52.6 44.0 7</td>
<td></td>
</tr>
<tr>
<td>NR9163 mutL</td>
<td>48.5</td>
<td>12.9</td>
<td>38.6 3</td>
<td></td>
</tr>
<tr>
<td>NR9066 mutD</td>
<td>12.0</td>
<td>16.8</td>
<td>71.1 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*dam methylation of viral (v) and complementary (c) strands (see Figure 1 and text).</td>
</tr>
</tbody>
</table>

Average of 3–7 determinations. The total number of plaques per determination averaged > 1000.

TC90, AA91, AA92, and AG92) are also well repaired. The others (TT87, TC87, AA88, AG88, TT90, and AG90) are poorly repaired, if at all. As expected, the mutL strain is generally deficient in mismatch repair, yielding between 50% and 60% mixed bursts regardless of the mismatch. With only one exception (see below), the mutD5 strain is as deficient as the mutL strain, mimicking mutL not only in the percentages of mixed bursts, but also in the ratio of white to blue plaques (data not shown, but very similar to the A-C-mismatch data of Table 2). The data are entirely consistent with the notion that the mutD-associated mismatch-repair deficiency resides in the mutHLS pathway of mismatch repair.

**mutD and VSP (Very Short Patch) repair:** The exception noted above to the identical behavior of mutD and mutL cells is the T-G mismatch at position 87: this mismatch is not repaired in the mutL strain but is significantly repaired in the mutD strain. The complete data for this mismatch are presented in Table 3 for me+/me+ and me+/me- configurations. The viral-strand sequence for this mismatch reads CTAGG (Figure 1) and the T-G mismatch here represents the special case of VSP (Very Short Patch) repair (Lieber, Allen, and Read 1986; Lieber 1987). VSP repair acts specifically at (wild-type) CCAGG sequences, where the second C in both strands is normally methylated by the dcm methylase yielding 5-methylcytosine. Deamination of 5-methylcytosine produces thymine instead of uracil and the lesion is therefore refractory to uracil-glycosylase repair. Unidirectional repair of the T-G mismatch to C-G by VSP repair remedies this situation. VSP repair requires the dcm, mutL, and mutS gene products, but no mutH or mutU (Lieber 1987), and operates independently of dam methylation. That this system operates at the (T-G)87 site in M13mp2 can be gleaned from the wild-type data in Table 3. Repair of T-G to C-G by VSP repair will produce a blue plaque. For the me+/me+ heteroduplex, the directionalities of VSP repair and mutHLS repair are
the same and a larger than normal excess of blue plaques is expected. For the me+ /me− heteroduplex, the two types of repair work in opposite directions and fewer white plaques are expected. Both expectations are fulfilled (for instance, compare the A-C data in Table 2 with the T-G data in Table 3).

**mutL** cells are deficient in both **VSP** and **mutHLS** repair (LIEB 1987; JONES, WAGNER and RADMAN 1987b; ZELL and FRITZ 1987) as confirmed by the data in Table 3. However, **mutD** cells repair the T-G mispair (only 7.7% to 12.0% mixed bursts). The directionality of this repair (excess of blue plaques) is the same regardless of methylation. These data strongly suggest that **mutD** cells are defective in the **mutHLS** pathway of repair but are fully proficient in the **VSP** repair pathway.

**Mismatch repair in double-mutator strains:** Further insights into the **mutD**-associated mismatch-repair defect may be obtained from studies with strains deficient in both the **mutD** and **mutHLS** pathways. Table 4 presents the results of heteroduplex transfections in a series of strains that includes the **mutDmutL** and **mutDmutS** double mutants. For two mismatches, i.e., (T-G)$_{87}$ and (A-C)$_{88}$ (the former being subject to **VSP** repair), **mutDmutL** and **mutDmutS** strains produce the same number of mixed bursts as the single **mutL** and **mutS** strains. Thus, adding the **mutD** defect does not further reduce the mismatch-repair deficiency in these strains.

**DISCUSSION**

In this paper I describe the nature of the mismatch-repair defect associated with **mutD** mutator strains of **E. coli**. The primary defect of **mutD** strains resides in the proofreading function of the Pol III holoenzyme, as demonstrated by both genetical and biochemical approaches. However, the high mutator strength of **mutD** (10$^4$- to 10$^5$-fold) casts doubt on this being the sole explanation for the mutator phenotype. It has been suggested that **mutD** and **dnaQ** mutators might have an additional defect in base selection (ECHOLS, LU and BURGERS 1983; PIECHOCKI et al. 1986). As an alternative, I suggested that **mutD** cells contain a defect in the **mutHLS**-dependent mismatch correction (SCHAAPER 1988). The data presented in this paper support this hypothesis.

Using the transfection of bacteriophage M13mp2 heteroduplex DNAs to assess the mismatch-repair capacity of various **E. coli** strains, we demonstrated the following: (1) **mutD**5 cells are essentially as deficient in mismatch repair as are **mutL** or **mutS** strains, (2) changes in methylation that markedly alter the directionality of repair in a wild-type strain, affect neither **mutD** or **mutL**, (3) small amounts of residual repair remaining in the **mutD**5 strain (when compared to the **mutL** strain) follow the directionality imposed by methylation, (4) throughout the range of different mismatches, the **mutD**5 strain remains as deficient as the **mutL** strain, whereas the wild-type strain displays the range of repair capacities characteristic of the **mutHLS** system, (5) strains doubly deficient in the **mutD** and **mutHLS** pathways are as unable to perform mismatch repair as are the single **mutD**, **mutL** or **mutS** strains. These combined results fully support the hypothesis that **mutD**5 cells are deficient in the **mutHLS**-dependent pathway of mismatch repair.

A number of mismatch-repair systems other than the **mutHLS** system are independent of **dam** methylation. The **VSP** repair pathway (LIEB 1987), directed at T-G mismatches in specific sequences, requires the **mutL** and **mutS** genes in addition to the **dcm** gene. It is further distinguished by its very short repair patch (10 nucleotides or fewer) in contrast to the **mutHLS** system whose repair tracts may extend over thousands of base pairs. The "localized repair" system described by RAPOSA and FOX (1987) appears similar to **VSP** and may, in fact, be identical. The present results show convincingly that the mismatch-repair deficiency of **mutD** strains does not include this type of repair. FISHEL, SIEGEL and KOLODNER (1986) have described a plasmid repair system dependent on the **mutS** and **uvrD** gene products. Some (minor) mismatch repair, fully independent of any **mutHLS** function (FISHEL and KOLODNER 1983; RAPOSA and FOX 1987), has also been described. In the present assay, 40–50% of all infective centers appear as pure bursts, even in **mutL** or **mutS** hosts, and these might result from a **mutHLS**-independent repair component. The general similarity between **mutD** and **mutL** strains leads us to conclude that such components are either minor, or, otherwise, independent of both **mutD** and **mutHLS**.

**TABLE 4**

<table>
<thead>
<tr>
<th>Mismatch</th>
<th>Strain</th>
<th>Genotype</th>
<th>Mixed</th>
<th>White</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T-G)$_{87}$</td>
<td>mut$^+$</td>
<td>3.3</td>
<td>52.6</td>
<td>44.0</td>
<td></td>
</tr>
<tr>
<td>mutL</td>
<td>48.5</td>
<td>12.9</td>
<td>38.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutS</td>
<td>45.8</td>
<td>5.7</td>
<td>48.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutD</td>
<td>12.0</td>
<td>16.8</td>
<td>71.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutDmutL</td>
<td>44.9</td>
<td>9.5</td>
<td>48.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutDmutS</td>
<td>45.8</td>
<td>5.7</td>
<td>48.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A-C)$_{88}$</td>
<td>mut$^+$</td>
<td>4.1</td>
<td>71.7</td>
<td>24.3</td>
<td></td>
</tr>
<tr>
<td>mutL</td>
<td>41.7</td>
<td>11.9</td>
<td>46.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutS</td>
<td>44.4</td>
<td>9.7</td>
<td>45.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutD</td>
<td>48.3</td>
<td>13.4</td>
<td>48.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutDmutL</td>
<td>47.5</td>
<td>10.6</td>
<td>41.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mutDmutS</td>
<td>46.8</td>
<td>10.8</td>
<td>42.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data represent measurements on hemimethylated (me$^+$/me−) molecules (v/c). Total plaques per measurement: >3000.

* Subject to **VSP** repair (see Table 3 and Figure 2).

* Data taken from Table 3.
Mismatch Repair in *E. coli* mutD

One other specific, methylation-independent repair system has recently been described, which is responsible for the unidirectional correction of A-G to C-G (Lu and Chang 1988; Su et al. 1988). A-G mismatches are thus subject to two types of repair: *mutHLS*-dependent repair correcting either half of the mismatch as instructed by the methylation bias, and the novel *mutHLS*-independent repair which removes the adenine regardless of methylation. Four examples of A-G mismatches are included in the present study (Figures 1 and 2). Two of them (GA<sub>60</sub> and AG<sub>92</sub>) are well repaired in the wild-type strain, the other two (AG<sub>66</sub> and AG<sub>69</sub>) only poorly. The observed repair of these mismatches appears to represent largely *mutHLS*-dependent repair, because it is abolished in *mutL* cells (Figure 2) and proceeds through preferential correction of the unmethylated, complementary strand. It appears that specific A-G repair does not significantly contribute to overall repair of the four A-G mismatches tested here. More detailed experiments, using molecules with various methylation patterns, might be required to address this question. Based on the present results, no indication is obtained that *mutD* might have an effect on this repair pathway.

Lundblad and Kleckner (1984) studied transposon Tn10 excision and discovered bacterial host mutations (called *tex*) that significantly enhanced the excision rate. The major class of *tex* mutations comprised strains affected in methylation-instructed mismatch correction: *dam*, *mutH*, *mutL*, *mutS* and *wrrD*. Surprisingly, among a series of other mutators tested, *mutD5* also enhanced excision. It was argued that this phenomenon was not likely related to high mutation rates *per se*, because no relationships could be detected between the strengths of these mutators and the enhancement of excision, and no effect was detected with other strong mutators, such as *mutT*. The present results, which place *mutD* in the class of the mismatch-repair defective strains, provide at least a partial answer to the question why *mutD* cells display enhanced transposon excision.

The reason why *mutD* cells are deficient in mismatch correction is not immediately obvious. Two possible hypotheses have been advanced (Schaaper 1988). First, the proofreading defect of *mutD* or *dnaQ* cells may product a high level of DNA replication errors, such that the mismatch-repair system becomes overloaded. This possibility has also been suggested by Radman et al. (1981) based on a heteroduplex transfection experiment with bacteriophage lambda. This hypothesis seems reasonable considering that the MutHLS proteins may be present in limited amounts in the cell (Lu et al. 1984). The second possibility is that the *mutD* (*dnaQ*) gene product, in addition to its proofreading function, plays a direct role in mismatch correction. It is noted that reconstitution of mismatch repair *in vitro* with purified proteins (Au et al. 1988) specifically requires Pol III holoenzyme (rather than Pol I). Until the detailed enzymology of mismatch repair is known (including the need for any exonuclease action), the possibility that mismatch repair might proceed in the absence of functional ε-subunit, must be considered.

In summary, the data in this paper demonstrate that *mutD* mutator strains of *E. coli* are severely compromised (>85% abolishment) in their ability to perform *mutHLS*-dependent mismatch repair. Since *mutHLS*-dependent mismatch repair contributes significantly (by several hundred fold) to preventing mutations in normal cells, this defect likely plays a major role determining the strong mutator effect of *mutD* strains.

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**LITERATURE CITED**


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