Repair of Single Base-Pair Transversion Mismatches of *Escherichia coli* in Vitro: Correction of Certain A/G Mismatches Is Independent of *dam* Methylation and Host *mutHLS* Gene Functions

A-Lien Lu and Dau-Yin Chang

Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201

Manuscript received December 17, 1987
Accepted January 7, 1988

ABSTRACT

Six different base-pair transversion mismatches are repaired with different efficiencies in an *in vitro* mismatch repair system. In particular, the T/T and C/C mismatches appear to be less efficiently repaired than the A/A and G/G mismatches. Four A/G and four C/T mismatches at different positions are repaired to different extents. One of the A/G mismatches is repaired equally efficiently when DNA heteroduplexes are fully methylated or hemi-methylated at the d(GATC) sequences. This type of mismatch repair appears to be unidirectional with A to C conversion by acting at A/G mispairs to restore the C/G pairs. This methyl-dependent correction is not controlled by the *mutH, mutL*, *mutS*, *uvrE, uvrB*, *phr*, *recA*, *recF*, and *recJ* gene products. The independence of the transversion mismatch repair of these genes and methylation distinguishes this from the known mismatch repair pathways.

DNA base-pair mismatches may arise from spontaneous replication error and homologous genetic recombination. In *Escherichia coli* mismatch repair directed by *dam* methylation at d(GATC) sequences is believed to correct errors arising during DNA replication (Claverys and Lacks 1986; Radman and Wagner 1986; Wagner and Meselson 1976). Repair is biased to the unmethylated newly synthesized DNA strand which bears the replication errors. The d(GATC) sequence are the activating sites for mismatch repair enzymes (Laengle-Rouault, Maenhaut-Michel and Radman 1986; Lahue, Su and Modrich 1987; Lu et al. 1984). The number and position of *dam* sites influence the repair efficiency (Lu 1987). This methyl-directed mismatch correction requires the products of genes *mutH*, *mutL*, *mutS*, and *uvrD* (or *uvrE* and *mutU*) both in vivo and in vitro (Bauer, Krammer and Knippers 1981; Lu, Clark and Modrich 1983; Nevers and Spatz 1975; Pukkila et al. 1983). Mismatch repair appears to involve long-patch excision and resynthesis (Wagner and Meselson 1976; Lu et al. 1984).

Mismatch repair in regions of heteroduplex DNA due to genetic recombination between complementary strands of two different parental molecules could be involved in gene conversion (Holiday 1964). The existence of a DNA mismatch repair system has been postulated in order to account for high negative interference (Norkin 1970; White and Fox 1974) and map expansion phenomena (Fincham and Holiday 1970; Holliday 1974). Such repair is thought to be independent of *dam* methylation and may be controlled by different gene products. Kolodner and co-workers (Fishel and Kolodner 1983; 1984; Fishel, Siegel and Kolodner 1986) have observed two methylation-independent pathways in *E. coli*. One pathway involved long excision tracts and does not require *mutH* or *mutL* function, but requires the *mutS* and *uvrD* gene products. The other very weak one associated with short repair tracts requires the *recF* and *recJ* gene products. A third pathway for mismatch repair in *E. coli* is characterized by very short repair tracts (rarely exceed ten nucleotides in length) (Lieb 1983; Lieb, Allen and Read 1986). This repair results in C to T transitions at the second position within the sequence 5’CC(A/T)GG, the *dam* recognition site. The repair acts to restore the G/C pair by replacing the thymine in the G/T mismatch and is apparently responsible for repairing deaminated 5-methylcytosines (Jones, Wagner and Radman 1987b). This short patch repair requires intact *mutL*, *mutS*, and *dam* genes but not *mutH* and *mutU* genes (Radman and Wagner 1986; Jones, Wagner and Radman 1987b).

In pneumococcus, it has been shown by DNA transformation that different types of base mismatches are processed differently by the *hex* controlled repair system (Claverys et al. 1981, 1983; Lacks, Dunn and Greenberg 1982). Similarly, the *E. coli* mismatch repair system does not recognize and repair all mismatches present in M13 (Kramer, Kramer and Fritz 1984) or lambda (Dohet, Wagner and Radman 1983; Wagner et al. 1984) with equal efficiency. In these experiments, heteroduplexes of
these two bacteriophages with single base-pair mismatches were used to transfect E. coli. The results with pneumococcus and E. coli are remarkably similar. In general, transition mismatches (G/T and A/C) are well repaired. Some transversion mismatches (A/G, C/T, and T/T) appear to be poor substrates for repair. It was noted that the repair of some transversion mispairs (especially A/G and C/T) depended on the neighboring nucleotide sequence (CLAVERYS et al. 1983; JONES, WAGNER and RADMAN 1987a; LACKS, DUNN and GREENBERG 1982). It is suggested that repair efficiency increases with increasing C/G content in the neighboring nucleotide sequences (JONES, WAGNER and RADMAN 1987a).

The in vitro assay for mismatch repair that has been developed (LU, CLARK and MODRICH 1983) is based on repair of heteroduplex DNA of fl R229, which contains a base-pair mismatch within the single EcoRI site of the molecule. Transition mismatches (G/T and A/C) are efficiently repaired in this in vitro system. The repair activity is dependent on the state of dam methylation of the DNA strands and on the gene functions of the mutH, mutL, mutS, uvrE, and ssb loci (LU, CLARK and MODRICH 1983; LU et al. 1984). The transversion mismatches have not been assayed in the in vitro system. In this paper, the specificity of E. coli mismatch repair mainly on the transversion mismatches was studied in the in vitro system. Similar results were obtained as in the in vivo systems, i.e., different transversion mismatches were repaired with different efficiencies. Repair of A/G and C/T mismatches was controlled by the flanking nucleotide sequences. It is the first demonstration that the repairable A/G mismatch was independent of dam methylation and did not require mutH, mutL, mutS, and uvrE gene products which are involved in methyl-directed mismatch repair. This methylation-independent correction of the A/G mismatch was quite efficient and was unidirectional with a conversion of A to C. This pathway appeared to be different from the other known methylation-independent pathways as judged by the requirement for known E. coli DNA repair gene products.

MATERIALS AND METHODS

Bacterial and bacteriophage strains: All E. coli strains used in this study are listed in Table 1. The bacteriophages fl used (Table 2) are derived from R229 (BOEKE 1981) which contains one EcoRI site at position 5616. Phages fl M28 and G18 containing a C to T substitution at position 5621 and T to G substitution at position 5620, respectively, were gifts from P. MODRICH. Other fl mutants were generated in this laboratory by oligonucleotide-directed mutagenesis as described below.

Other materials: Restriction enzymes BamHI, BanII, DpnI, HincII, MboI and Sau3A, large fragment of DNA polymerase I and T4 DNA ligase were from Bethesda Research Laboratories. Endonuclease BsmI was from New England Biolabs. Endonucleases EcoRI and BspI and dam methylase were from P. MODRICH. Oligonucleotides ALLC1 (CGCGAAGTCCGGCGA) and ALLC2 (CCGAAATCCGGCGA) were purchased from the DNA Synthesis Facility. University of Massachusetts Medical School. Oligonucleotides ALLC3 (GAAAGCTTATTCCG), ALLC4 (GAAAGCGGCGCGCG), ALLC7 (CCGATTTGGCGGCGGAA), and ALLC8 (AAAGCCGATTTCCGCG) were made with an Applied Biosystems DNA synthesizer by D. SITTMAN at the University of Mississippi. S-[methyl-3H]Adenosyl methionine (15.8 Ci/m mole) and [α-
Closed heteroduplex of fl R229 yields 3.0-kb and 3.4-kb fragments upon hydrolysis with EcoRI. This generates the EcoRI restriction site and renders the site sensitive to mismatch at position 5620 (represented by 5620 of fl DNA). The substrate for mismatch repair is a covalently closed DNA containing the (GAATTC) recognition sequence. Mismatch correction on viral strand causes the complementary strand containing the EcoRI recognition site and 2.5-kb and 3.9-kb fragments with EcoRI site as described (Lu 1987). After transfection, methylated heteroduplexes were constructed (Lu, CLARK and MODRICH 1983) and analyzed by DNA sequencing.

**RESULTS**

**Repair of transversion base mismatches**: DNA substrates containing transversion mismatches were studied in vitro for mismatch repair. Hemi-methylated heteroduplexes were prepared from fl R229 (containing the EcoRI sequence) and eight different fl mutants which contain a single nucleotide change within the EcoRI recognition site (Figure 1) and analyzed by electrophoresis on 1% agarose gel (Lu, CLARK and MODRICH 1983). The purified DNA products were digested with EcoRI and BamHI (for detection of generation of EcoRI site) or with BsmI (for detection of generation of BsmI site) (Figure 1) and analyzed by electrophoresis on 1% agarose gel (Lu, CLARK and MODRICH 1983).

---

**Oligonucleotide-directed mutagenesis**: Oligonucleotides (15 bases) of ALLC 1, 2, 3, 4, 7, and 8, which are complementary to the fl viral sequence in the intergenic region, were used to generate phage mutants without the EcoRI site, to a form which is subject to cleavage by EcoRI endonuclease. In this case correction can be detected by only one type of conversion in which the EcoRI mutant strand is repaired to EcoRI containing strand. The heteroduplexes containing an A/G or C/T mismatch at position 5620 have one strand containing the BsmI recognition sequence and the other strand containing the EcoRI recognition site (Table 2). Six possible single base-pair transversion mismatches can be constructed within the EcoRI site of the fl heteroduplexes. The hybrid EcoRI site which is resistant to EcoRI cleavage can be converted to an EcoRI-sensitive form when the strand bearing the mutan EcoRI is repaired with cell extract. Hemi-methylated DNA prepared by using methylated RF and unmethylated viral strands was resistant to cleavage by MboI, indicating that all four d(GATC) sites were in the hemi-methylated state. However, as judged by sensitivity to MboI endonuclease, the methylation of viral strands was about 90% complete at each d(GATC) sequence (Lu, CLARK and MODRICH 1983) in molecules prepared from unmethylated RF and methylated viral strands (prepared from K38 harboring pGG503, a dam overproducer) (HERMAN and MODRICH 1982). The results of repair with A/A, T/T, G/G, and C/C mismatches are presented in Table 3 and with A/G and C/T mismatches in Table 4. For comparison, a G/T transition mismatch was repaired at an efficiency of 42% (Table 5, line 1) under the same assay conditions. The data in Tables 3 and 4 indicate that different transversion mismatches are not re-

---

**FIGURE 1.**—In vitro repair assay for A/G mismatch at position 5620 of fl DNA. The substrate for mismatch repair is a covalently closed heteroduplex of fl R229 (Boere 1981) containing a A/G mismatch at position 5620 (represented by ρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρρrho
TABLE 3

<table>
<thead>
<tr>
<th>Heteroduplex</th>
<th>Mismatch position</th>
<th>Mismatch</th>
<th>State of repair</th>
<th>EcoRI site methylated</th>
<th>EcoRI site repaired (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5618</td>
<td>GAATTC</td>
<td>Me+</td>
<td>43</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5620</td>
<td>GAATTA</td>
<td>Me-</td>
<td>40</td>
<td>CTTCAG</td>
<td></td>
</tr>
<tr>
<td>5618</td>
<td>GAATTC</td>
<td>Me-</td>
<td>11</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5620</td>
<td>GAATTC</td>
<td>Me+</td>
<td>8</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5621</td>
<td>GAATTC</td>
<td>Me-</td>
<td>37</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5616</td>
<td>GAATTC</td>
<td>Me+</td>
<td>19</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5621</td>
<td>GAATTC</td>
<td>Me-</td>
<td>6</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5616</td>
<td>GAATTC</td>
<td>Me+</td>
<td>4</td>
<td>CTTAAG</td>
<td></td>
</tr>
</tbody>
</table>

* Results represent the average of at least three experiments.

TABLE 4

<table>
<thead>
<tr>
<th>Heteroduplex</th>
<th>Mismatch position</th>
<th>Mismatch</th>
<th>State of repair</th>
<th>EcoRI site methylated</th>
<th>Percent DNA being repaired to EcoRI (E) or BsmI (B) site</th>
</tr>
</thead>
<tbody>
<tr>
<td>5618</td>
<td>GAATTC</td>
<td>Me+</td>
<td>10</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5621</td>
<td>GAATTA</td>
<td>Me-</td>
<td>47</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5620</td>
<td>GAATTC</td>
<td>Me-</td>
<td>36</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5621</td>
<td>GAATTC</td>
<td>Me+</td>
<td>43</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5618</td>
<td>GAATTC</td>
<td>Me-</td>
<td>51</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5621</td>
<td>GAATTC</td>
<td>Me+</td>
<td>19</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5620</td>
<td>GAATTC</td>
<td>Me-</td>
<td>24</td>
<td>CTTAAG</td>
<td></td>
</tr>
<tr>
<td>5621</td>
<td>GAATTC</td>
<td>Me+</td>
<td>19</td>
<td>CTTAAG</td>
<td></td>
</tr>
</tbody>
</table>

* Results represent the average of at least three experiments.

Table 3 describes the in vitro repair of A/A, T/T, G/G, and C/C mismatches. The table shows the repair efficiency for different mismatches at various positions, with some mismatches being more efficiently repaired than others. The repair efficiency is indicated by the state of repair (e.g., Me+ or Me-) and the percentage of DNA repaired in the EcoRI site.

In Table 4, the in vitro repair of A/G and C/T mismatches at different positions is evaluated. This table shows the repair efficiency for A/G and C/T mismatches at various positions, with some mismatches being more efficiently repaired than others. The repair efficiency is indicated by the state of repair (e.g., Me+ or Me-) and the percentage of DNA repaired in the EcoRI site.

In both tables, the results represent the average of at least three experiments. The repair of A/A, T/T, G/G, and C/C mismatches appears to be more efficient than A/G and C/T mismatches. The repair efficiency is affected by the position of the mismatches, with some mismatches being more efficiently repaired at different positions.

In Table 5, the independence of A/G mismatch repair on dam methylation and mutH and mutS gene products is evaluated. This table shows the repair efficiency for A/G mismatches at different positions, with some mismatches being more efficiently repaired than others. The repair efficiency is indicated by the state of repair (e.g., Me+ or Me-) and the percentage of DNA repaired in the EcoRI site.

The results represent the average of at least three experiments. The repair of A/G mismatches at different positions is influenced by the presence of dam methylation and mutH and mutS gene products. The repair efficiency is affected by the state of methylation and the presence of the gene products.
match at position 5620. The results shown in Table 6 (see below) indicate that correction on the two strands may occur via different mechanisms (one is dependent and the other is partially dependent on methylation). Therefore, the repair of A/G and C/T transversion mismatches may be controlled by neighboring sequence environment as well as orientation relative to the methylated DNA strand.

Effect of *E. coli* mutations and dam methylation on transversion mismatch repair: Strains defective in the *mutH*, *mutL*, *mutS*, and *uvrE* genes are deficient in methyl-directed mismatch repair (BAUER, KRAMMER and KNIPPERS 1981; LU, CLARK and MODRICH 1983; NEYERS and SPATZ 1975; PUKKILA et al. 1983). The *mutH* gene product contains a specificendonuclease activity which nicks 5' to the dG of d(GATC) sequences on unmethylated DNA strand (WELSH, LU and MODRICH, 1986; WELSH et al. 1987). It is believed that MutH protein is responsible for the strand discrimination on dam-dependent mismatch repair (LAENGLE-ROUAULT, MAENHAUT-MICHEL and RADMAN 1987). Therefore, repair reactions were performed with *mutH* cell extract for the repairable transversion mismatches (A/A, G/G, A/G, and C/T). The *in vitro* mismatch correction occurred poorly in the *mutH* extract for the A/A and G/G mismatches (data not shown) as in the case of a G/T mismatch (LU, CLARK and MODRICH 1983) (Table 5). The repair of the C/T mismatch at position 5618 was also dependent on *mutH* gene function. The repair efficiency was reduced from 51% to 4% when *mutH* extract was used instead of wild type extract. However, the A/G mismatches at position 5621 were well repaired in *mutH* extract (Table 5). The *mutH*-independent repair of the A/G mismatch indicates that it is also methylation-independent. The data in Table 5 confirm the conclusion that this A/G mismatch was repaired equally efficiently when DNA heteroduplexes were fully methylated or hemi-methylated at the d(GATC) sequences. The *mutS* gene product which binds specifically to the mismatched site (SU and MODRICH 1986) is involved in dam-dependent mismatch repair as well as two dam-independent repair pathways (FISHEL and KOLODNER 1983; 1984; RADMAN and WAGNER 1986). However, the *mutS* mutant extract has the capacity to repair the A/G mismatch at position 5621 (Table 5). In comparable assays (Table 5), the G/T mismatch was not repaired in *mutH* or *mutS* cell extract or when both DNA strands were fully modified by the dam methylase (HERMAN and MODRICH 1982) in a wild-type extract.

The other repairable A/G mismatch at position 5620 (Table 4, lines 3 and 4) was also tested for the dependence of repair on methylation and the products of *mutH* and *mutS* loci. As mentioned before, heteroduplexes with this A/G mismatch have the viral strand containing the *BsmI* recognition sequence and the complementary strand containing the EcoRI recognition site (Figure 1). After repair *in vitro* the DNA samples were divided into two halves: one was cut with EcoRI and *BamH*II and the other was digested with *BsmI*. Mismatch repair was measured as the percentage of DNA repaired to generate an EcoRI or *BsmI* site. Repair of G to T that generates the EcoRI site on both DNA strands was dependent on both the *mutH* and *mutS* gene products and also dam methylation (Table 6, column 3). Repair of A to C that generates the *BsmI* site was partially dependent on *dam* methylation and the *mutH* and *mutS* gene products (Table 6, column 4). There is about 20% of the DNA which is repaired to a *BsmI* site when both DNA strands were fully methylated or in the *mutH* and *mutS* cell extracts in the other three states of methylation. The results summarized in Tables 5 and 6 indicate that the *mutS*-independent correction of an A/G mismatch is unidirectional with an A to C conversion.

Other *E. coli* mutant extracts were tested for this methylation-independent pathway. The gene products of *mutL* and *uvrE* that are also involved in the *dam*-dependent mismatch repair had no effect on this type of mismatch correction (data not shown). The methylation-independent pathway described in this paper did not require the *recF* and *recJ* gene products (data not shown) which have been shown to be involved in one of the methylation-independent

---

### TABLE 6

<table>
<thead>
<tr>
<th>mutS-independent repair of A/G mismatch is unidirectional with A to C conversion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5616</td>
</tr>
<tr>
<td>5621</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>State of methyltion (v/c)*</th>
<th>Cell extract</th>
<th>Percent DNA being repaired to</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Me+/Me- WT'</td>
<td>11</td>
<td>56&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. Me+/Me- mutH</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>3. Me+/Me- mutS</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>4. Me+/Me+ WT</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>5. Me-/Me+ WT</td>
<td>43&lt;sup&gt;3&lt;/sup&gt;</td>
<td>19</td>
</tr>
<tr>
<td>6. Me-/Me+ mutH</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>7. Me-/Me+ mutS</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>8. Me+/Me+ WT</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>9. Me-/Me- WT</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>10. Me-/Me- mutH</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>11. Me-/Me- mutS</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

* Results represent the average of at least three experiments.  
<sup>1</sup> Viral/complementary.  
<sup>2</sup> Wild type.  
<sup>3</sup> These data were presented in Table 4.  
<sup>a</sup> DNA derived from Me+/Me- heteroduplex by *in vitro* methylation by purified dam methylase.  
<sup>e</sup> Same as in footnote <sup>e</sup> but derived from Me+/Me+ DNA.
pathways (Fishel and Kolodner 1983, 1984). Moreover, the major recombination control genes, recA, and thymine dimer repair genes, phr, and uvrB, had no effect either. Therefore, this type of repair is a novel pathway which is different from the three characterized dam methylation-independent pathways (Fishel and Kolodner 1983, 1984; Fishel, Siegel and Kolodner 1986; Lieb 1983; Lieb, Allen and Read 1986; Radman and Wagner 1986) because they require different sets of gene products. So far, only ssb (gene for single-stranded DNA binding protein) mutation of host genes tested had some effect on this dam-independent repair. Repair with an extract prepared from an ssb mutant was 40% of that observed with wild-type extract.

**DISCUSSION**

The efficiency of mismatch repair could be controlled by two factors: the structure of the mismatch itself and the neighboring sequence of the mismatched base. Six possible single base-pair transversion mismatches within f1 DNA were compared in an in vitro assay. A cell-free system provides attractive possibilities for analysis of the molecular basis of mismatch specificity. The data presented in this paper show that the E. coli in vitro mismatch repair system does not repair all mismatches with equal efficiency. The results are remarkably similar to the in vivo systems using M13 (Kramer, Kramer and Fritz 1984) and lambda phages (Dohet, Wagner and Radman 1985; Wagner et al. 1984). The T/T and C/C mismatches appear to be less repaired than A/A and G/G mismatches. The A/G and C/T mismatches are repaired at various efficiencies primarily due to the neighboring sequence environment.

The difference of sequence context was minimized by narrowing the mismatches within the six bases of the EcoRI recognition sequence. Nevertheless, sequence environment still has an effect on repair efficiency. The A/A, T/T, and C/C mismatches at two different positions showed little difference in repair efficiency. However, the repair of A/G and C/T mismatches was strongly influenced by the flanking sequences (Table 4). This is consistent with the results of Jones, Wagner and Radman (1987a) but the rule that the repair efficiency increases with increasing C/G content in the neighboring sequence seems not to apply in this case. The repair of an A/G mismatch at position 5618 is less efficient than at position 5621, however the number of C/G pairs of 4 base pairs on either side of the mismatch is 5 for position 5618 and is 3 for position 5621.

The methylation-independent repair of the A/G mismatch was observed at positions 5620 and 5621. At position 5621, the A/G mismatch was repaired in mutH or mutS extracts to the same extent as in the wild type extract and the repair was independent of dam methylation (Table 5). However, at position 5620, only 50% of the total repair is independent of dam methylation. There are always about 20% of input DNA showing A to C conversion which is independent of dam methylation and mutH and mutS gene products (Table 6). The G to T conversion on the viral strand is dependent on dam methylation and mutH and mutS gene products. The mutS-independent repair, i.e., the repair in the mutS cell extract, is predominantly on A to C conversion at complementary DNA strands (19% vs. 4%).

The dam- and mutS-independent pathway described here is characterized by several properties. First, this pathway can be as efficient in extent of repair as is the dam-dependent pathway. Second, this repair is specific for an A/G mismatch and causes an A to C conversion. At position 5620, the methylation-independent repair is biased to A to C conversion but not G to T conversion (Table 6). At position 5621, repair was scored as the conversion of A to C on the viral strand that generates the EcoRI site (Table 5). Conversion of G to T on the complementary strand can not be detected due to the limitation of the in vitro assay (the lack of a restriction site generated). But, when heteroduplex DNA was transformed into wild-type or mutS mutant E. coli cells, this A/G mismatch was preferentially corrected to a C/G pair (our unpublished results). The A to C conversion of an A/G mismatch found in f1 DNA at positions 5620 and 5621 was also observed in Strep-tococcus pneumoniae in which the system acts upon 5' ... ATTAAT ... 3'/3' ... TAAGTA ... 5' (Sicard et al. 1985). It is interesting to point out that in all three cases the A/G mismatched bases are surrounded by clusters of at least three A/T pairs. These structures may be recognized by a specific enzymatic system because the A/G mismatch of f1 DNA was not efficiently repaired at position 5618 which lacks the runs of A/T on one side (Table 4). The A/G mismatch located within a short palindrome may be the other common feature shared by this conversion. Third, it requires a different set of E. coli gene products from the three known methylation-independent ones (Fishel and Kolodner 1983, 1984; Fishel, Siegel and Kolodner 1986; Lieb 1983; Lieb, Allen and Read 1986; Radman and Wagner 1986). Two of the dam-independent repair pathways require the mutS gene function and the other one requires the recF and recJ gene products. The specific pathway for A to C conversion described here was not controlled by the mutH, mutL, mutS, uvrE, uvrB, phr, recA, recF, and recJ gene products. Therefore, this dam- and mutS-independent repair pathway appears to be novel. Attempts to identify host genes that control this A to C conversion were unsuccessful. The only gene product tested so far which showed an influence on this
methylation-independent pathway is single-stranded DNA binding protein whose general properties have been described (CHASE and WILLIAMS 1986).

The dam-dependent mismatch repair of *E. coli* is accompanied by repair DNA synthesis which occurs predominantly on the unmethylated strand and is localized near d(GATC) sequences (LU, CLARK and MODRICH 1983; LU et al. 1984). To characterize the repair synthesis for the novel mutS- and methylation-independent pathway, similar experiments were performed (data not shown) to detect specific incorporation of 32P-nucleotide precursor into restriction fragments. Reaction products after incubation with cell extract in the presence of [α-32P]dATP were isolated and hydrolyzed with BspI and BanI restriction endonucleases. The A/G mismatch at position 5621 is repaired to the same extent in mutS and wild-type extracts. However, the DNA repair synthesis patterns are quite different under these conditions (our unpublished results). Repair DNA synthesis was localized to fragments containing the d(GATC) sequences in the wild type but not in the mutS extract. The repair synthesis pattern in the wild-type extract is characteristic of the methylation-dependent pathway and is similar to the one reported by LU et al. (1984). We cannot detect any localized incorporation for the new pathway in mutS extract even repair occurs under this condition. It is possible that the repair tract is too short to be evident. However, the repair pattern of this dam- and mutS-independent pathway is clearly different from the one of dam-dependent pathway. It is likely that both methylation-dependent and-independent repair pathways are operating in the wild type cell extract with hemi-methylated DNA containing an A/G mismatch at position 5621. The non-additivity of the two repair systems however suggests that some components are shared or limiting for these two types of repair.

The dam-independent nature of the A to C conversion at an A/G mismatch suggests that this type of mismatch repair in *E. coli* is not functional in error avoidance during DNA replication. Because the repair of A/G mismatches is unidirectional with A to C conversions, replicative errors inserting adenines opposite the guanines will be corrected and errors inserting guanines opposite the adenines will be preserved. This may reduce the transversion frequency of C/G to A/T and increase the frequency of A/T to C/G transversions. The A to C conversion may be involved in the gene conversion in regions of heteroduplex DNA formed during genetic recombination.

This repair process could account for many earlier findings showing high negative interference (NORKIN 1970; WHITE and FOX 1974) or map expansion (FINCHAM and HOLLIYDAY 1970; HOLLIYDAY 1974). The hyperrecombination observed in some λ crosses (LIES 1983) and pneumococcal transformation (SI-CARD et al. 1985) is caused by localized conversion through short-patch mismatch repair (RADMAN and WAGNER 1986).

Recent work in this laboratory has focused on the identification and characterization of proteins that mediate this specific A/G mismatch repair. Extracts of *E. coli* cells have been found to contain a protein that binds specifically to duplexes containing A/G mismatches rather than T/G mismatches or to homoduplexes (A.-L. LU and D.-Y. CHANG, unpublished results). A specific endonuclease was associated with the A/G mismatch binding protein. These two activities co-purify through two chromatographic steps. Elucidation of the mechanism of this reaction requires identification of genes involved and its reconstitution in a purified system.

The authors thank L. BLACK and P. WOLFE for helpful criticism of the manuscript. Special thanks go to PAUL MODRICH and JOHN HAYES for providing bacterial strains and fl mutant phages. This work was supported by grant GM 35132 from the National Institute of General Medical Sciences.

**LITERATURE CITED**


JONES, M., R. WAGNER and M. RADMAN, 1987a Repair of a mismatch is influenced by the base composition of the surrounding nucleotide sequence. Genetics 115: 605–610.