provides evidence for a loop repair pathway in mismatch repair genes. This activity is distinct from the MutHLS mismatch repair pathway. Strand specificity and repair efficiency are largely independent of the GATC methylation state of the DNA and presence of the products of mismatch repair genes muH, muL, and muS. This study provides evidence for a loop repair pathway in E. coli that is distinct from conventional mismatch repair.

DNA mispairs can occur within the DNA helix as a consequence of DNA biosynthetic errors or as a result of recombinational strand transfer between nonidentical sequences (1–4). Such pairing errors may take the form of base-base mismatches or loops, in which one strand contains a single stranded loop. Specific correction of these base-base mismatches by the system requires Mg²⁺ and the four dNTPs and is efficiently carried out on insertions and deletions. This activity is distinct from the MutHLS mismatch repair pathway. Strand specificity and repair efficiency are largely independent of the GATC methylation state of the DNA and presence of the products of mismatch repair genes muH, muL, and muS. This study provides evidence for a loop repair pathway in E. coli that is distinct from conventional mismatch repair.

The directed DNA repair efficiency of a set of M13mp18-derived heteroduplexes containing 8-, 12-, 16-, 22-, 27-, 45-, and 429-nucleotide loops was determined by in vitro assay. Unpaired nucleotides of each heteroduplex reside within overlapping recognition sites for two restriction endonucleases, permitting independent evaluation of repair occurring on either DNA strand. Our results show that a strand break located either 3’ or 5’ to the loop is sufficient to direct heterology repair to the nicked strand in Escherichia coli extracts. Strand-specific repair by this system requires Mg²⁺ and the four dNTPs and is equally efficient on insertions and deletions. This activity is distinct from the MutHLS mismatch repair pathway. Strand specificity and repair efficiency are largely independent of the GATC methylation state of the DNA and presence of the products of mismatch repair genes muH, muL, and muS. This study provides evidence for a loop repair pathway in E. coli that is distinct from conventional mismatch repair.

DNA mispairs can occur within the DNA helix as a consequence of DNA biosynthetic errors or as a result of recombinational strand transfer between nonidentical sequences (1–4). Such pairing errors may take the form of base-base mismatches or loops, in which one strand contains one or more unpaired nucleotides. Strand-specific correction of base-base mismatches produced during DNA biosynthesis plays an important role in mutation avoidance (2, 5, 6), and mismatch repair within the recombination heteroduplex has been implicated in gene conversion (3, 4, 7, 8).

Base-base mispairs are subject to strand-specific correction by the mismatch repair system of both prokaryotes and eukaryotes (5, 6, 9, 10), but action of this system on loop mismatches is limited to fairly small heterologies. The Escherichia coli mismatch repair pathway will correct loops up to about 7 unpaired nucleotides, but larger heterologies are poorly processed by this system (11–14). A similar specificity is characteristic of the human mismatch repair system, which can correct loops up to about 10 unpaired nucleotides (15–18).

There is evidence that eukaryotes can rectify large unpaired heterologies by a pathway distinct from the mismatch repair system. Available evidence suggests that specificities of the eukaryotic mismatch repair and large loop repair systems partially overlap. When transformed into Saccharomyces cerevi-
partially complementary to the sequence between XhoI and XhoI sites of M13LR8. Mutant M13LR8 phages were identified by restriction analysis of replicative form minipreparation and then confirmed by DNA sequencing. The set of M13LR derivatives produced in this manner allowed preparation of G-T mismatch and 8-, 12-, 16-, 22-, 27-, 45-, and 429-nucleotide insertion/deletion heteroduplexes (see Table I). Unmethylated phage DNAs were isolated from RS5033, whereas fully methylated phage replicative form DNAs were isolated from NM522.

Construction of Heteroduplex DNA—Heteroduplex DNA substrates were constructed essentially under the conditions of Lu et al. (28). GATC-methylated M13LR derivative replicative form DNA (1 mg) was linearized with BglII or EcoRI and mixed with a 4-fold molar excess of unmethylated viral DNA, followed by alkaline denaturation and annealing. After isolation by hydroxyapatite chromatography, double strand linear homoduplex DNA was removed by treatment with ATP-dependent DNase as described (29). The open circular heteroduplex was purified by Sephadex G-200 (Sigma) chromatography and benzoylated naphthylphosphatase DEA-cellulose (Sigma) chromatography in 10 mM Tris-HCl (pH 7.6), 1 mM NaCl, 1 mM EDTA (14). The strand break generated by EcoRI was about 49 bp 3’ to the heterology for the loop size greater than 22 nucleotides and about 70 bases 3’ to the smaller loops. A nick generated by BglII was about 150 bp 5’ to the loops. The several-bp variation in the nick heterology distance for the different heteroduplexes is a consequence of the presence of different restriction site markers in the different heterologies.

Covalently closed DNA was ligated with E. coli DNA ligase in the presence of ethidium bromide (96 mmol of dye/mol of nucleotide) and isolated by equilibrium centrifugation in CsCl/ethidium bromide (28). Substrates containing fully methylated GATC sites were prepared by treatment of hemimethylated heteroduplex with Dam methylase as described (28).

Substrates used in this study are summarized in Table I and Fig. 1. By pairing different M13LR insertion derivatives, heteroduplexes containing base-base mismatch or distinct site-specific insertions/deletions were constructed. Extrahelical segments can be located within either viral or complementary strand.

Repair Assays—Growth of cells and preparation of cell extracts were as described by Lu et al. (28). Repair in concentrated E. coli lysate was carried out in 10-μl reactions containing 0.02 mM Tris-HCl (pH 7.6); 5 mM MgCl2; bovine serum albumin at 50 μg/ml; 1 mM ATP; 0.1 mM each dATP, dGTP, dCTP, and dTTP; and 0.1 μg (21 fmol) of heteroduplex DNA. The optimal concentration of E. coli extracts was 7–10 mg of protein/ml. After incubation at 37 °C for 1 h, reactions were terminated by adding 50 μl of 25 mM EDTA (pH 8.0), and DNA was purified by phenol extraction and ethanol precipitation. The DNA was then analyzed by restriction endonuclease digestion and agarose gel electrophoresis. DNA products were quantitated after ethidium staining using a gel documentation CCD camera (UVP Ltd.) (14, 30).

RESULTS

Construction of Heteroduplexes in Which the Large DNA Heterologies Reside in a Similar Sequence Environment—Starting from phage M13mp18, we have prepared a set of M13LR derivatives (14) that contain extra nucleotides within the polylinker region located between the single EcoRI and HindIII sites of M13mp18. This set of M13LR derivatives permits construction of heteroduplexes representing base pair mismatches and 8-, 12-, 16-, 22-, 27-, 45-, and 429-nucleotide insertion/deletion heterologies (Fig. 1 and Table I). The heterology in each of these heteroduplexes is located within a similar environment. Moreover, as shown in Fig. 1, each insertion/deletion mismatch is located within overlapping restriction endonuclease recognition sites, permitting independent evaluation of correction on either DNA strand. Digestion of the heteroduplex DNA with AlwNI (Fig. 1) and the indicator restriction endonuclease, whose recognition site is blocked by the presence of the heterology (14), will yield a 7.2-kb fragment only. Similar digestion of DNA in which the recognition sequence has been restored by repair will yield 4.1- and 3.1-kb fragments. All substrates used here were refractory to the digestion by the indicator restriction endonucleases in the absence of repair (14) (data not shown).

GATC methylation state dictates the strand specificity of E. coli MutHLS dependent mismatch repair pathway (6). Thus, we also constructed large loop substrates of different methylation states to evaluate the effect of this system. The M13mp18 DNA contains six d(GATC) sites recognized by dam methylase (positions 1392, 1714, 6253, 6406, 6502, and 6936). The map also shows restriction endonucleases sites used to generate strand break on complementary strand in this study.

Each substrate was a circular heteroduplex derived from bacteriophage M13mp18 and containing a single-strand loop located within overlapping recognition sites for two restriction endonucleases near the multiple cloning site. In the example shown, a 22-nucleotide loop of the viral DNA strand (V22) resides within overlapping sequences recognized by HindIII and XhoI endonucleases. Although the presence of the loop renders this site resistant to cleavage by either endonuclease, repair occurring on the complementary (C) DNA strand fills in the loop and generates an XhoI-sensitive site, whereas correction on the viral (V) strand results in the loop removal and HindIII sensitivity. The DNA of M13mp18 contains six d(GATC) sites recognized by dam methylase and dam methylase-proficient strain. The fully methylated C-strand was prepared from the purified phage grown in dam–E. coli strain, and the methylated C+ strand was prepared from the RF DNA grown in Dam methylase-proficient strain. The fully methylated substrates were also prepared and analyzed.

Although the E. coli mismatch repair system is able to repair small insertion/deletion mismatches in a methyl-directed, strand-specific manner (14), the activity of this system on such structures appears to be restricted to heterologies containing less than about seven unpaired nucleotides (14). Although strand breaks are known to be required for efficient correction by eukaryotic loop repair systems (6), a potential activation role for strand discontinuities in large heterology repair in E. coli has not been tested. In order to clarify the nature of this reaction, we have constructed a set of circular heteroduplexes in which the nick was located at different positions to evaluate the potential role of strand-specific single-strand breaks in the processing of such structures. We designate these heteroduplexes according to the DNA strand containing the unpaired segment, the number of unpaired nucleotides in the heterology, and placement of the strand break 3’ or 5’ to the heterology as viewed along the shorter path joining the two DNA sites in the circular DNA.
TABLE I

| DNA   | V  | C  | Markers          |
|-------|----|----|------------------|
| GT    | LR1|    | XhoI             |
| C8    | LR3|    | HindIII         |
| C12   | LR10|   | NcoI            |
| V12   | LR15|   | EcoRV           |
| C16   | LR12|   | XhoI            |
| V16   | LR15|   | EcoRV           |
| C22   | mp18|   | HindIII         |
| V22   | LR1 |   | XhoI            |
| C45   | mp18|   | HindIII         |
| V45   | LR2 |   | XhoI            |
| V429  | LR4 |   | HindIII         |

Nick-directed Repair of Large DNA Heterologies in Vitro—Heteroduplexes containing large insertion/deletion heterologies and a site-specific strand break were tested for repair in E. coli cell-free extracts. Table II compares the efficiency of correction of all heteroduplexes as scored by restriction endonuclease digestion, and Fig. 2 illustrates typical results obtained with the restriction assay.

It is known that a single-strand break located either 3' or 5' to a mispair is sufficient to provide strand specificity for MutHLS-dependent mismatch repair (6). In order to avoid MutHLS-dependent activity complicating our analysis, we choose to test extracts from a mismatch repair-deficient RK1517 mutS strain. As shown in Table II, unpaired heterologies of 22 nucleotides located in the complementary strand or viral strand of a covalently closed circular heteroduplexes were subject to limited processing in both mismatch-proficient and -deficient cell extracts (Table II, CC-circle). By contrast, unpaired heterologies of various size ranges were efficiently rectified in open circular DNAs containing a nick about 49 or 70 base pairs 3' to the loop, or about 150 base pairs 5' to the unpaired region in the 7.2-kilobase pair circular heteroduplexes (Fig. 2 and Table II). In reactions containing the MutS-deficient extract, repair of these DNAs displayed a substantial bias (4-40-fold) toward the incised DNA strand, although significant repair was detected on the closed DNA strand in some cases (Table II; V-entries of V45 and V429). A G-T mismatch was used as a positive control for methyl-directed mismatch repair. As shown in Table II, the efficiency of nick-directed correction of these large heterologies was 50–180% of that observed for a 3'-G-T mismatch on the unmethylated strand when extracts were derived from the methyl-directed repair-proficient NM522 cells.

Whereas all of the 3'- and 5'-heteroduplexes used in this study contained the strand break in the complementary strand, the substrates included several with the unpaired heterology present in either the complementary or viral strand (Table II).
ATP is essential for the MutHLS pathway (6). However, we cannot exclude the possibility that the dATP used for repair resynthesis may substitute for ATP required in the reaction.

**Genetic Independence of Nick-directed Loop Repair from Mismatch Repair**—Methyl-directed mismatch repair is dependent on the product of mutH, mutL, and mutS genes. In addition, MutHLS have been implicated in the repair of 1-7-base deletion heteroduplexes (12, 14). Consequently, cell extracts were prepared from *E. coli strains* that were defective in each of these gene products and were assayed with large nucleotide insertion/deletion heteroduplexes described above. As shown in Table II, correction of large nucleotide heterologies is independent of the presence of the mutS gene product in crude extracts. Large loop repair is also independent of the mismatch genes mutL and mutH, as deduced from assays using extracts from a mutant strain (Table V). Correction was assayed using the nicked C12, V22, C45, and V429 substrates. Loop repair on nicked strand occurred at similar levels in extracts from nicked C12, V22, C45, and V429 substrates. Loop repair on the complementary strand; V−, repair occurring on the viral strand. Repair shown for each DNA strand is the average of at least three measurements ± S.D. Complete repair would correspond to 21 fmol.

**Fig. 2.** Repair of heteroduplex containing a mismatch or loop heterologies in *E. coli* extracts. Repair reactions with *E. coli* strains RK1517 (mutS) and NM522 (mismatch repair-proficient) cell-free extracts were performed as described under “Experimental Procedures.” DNA products were digested with AluN1 and the appropriate restriction endonuclease (Table I) and then subjected to agarose gel electrophoresis to score loop correction occurring on each DNA strand. **Upper two panels,** reaction of 3'-heteroduplexes with a nick at the EcoRI site, about 49 or 70 bases 5' to the loops. **Lower two panels,** reaction of 5'-heteroduplexes with a nick at BglII site, about 150 bases 5' to the loop. Repair occurring on the continuous unmethylated V strand and the open methylated C strand is indicated. The bar pointing to the 7.2-kb fragment represents unrepaird substrates; bars pointing to 4.1- and 3.1-kb fragments indicate corrected products. In the case of the V429 heteroduplex, repair on the C strand yielded 4.1- and 3.5-kb fragments.

### Table II

**Efficiency of loop repair in *E. coli* extracts**

| Substrates | Repair | C+ | V− | C+ | V− |
|------------|--------|----|----|----|----|
| CC-Circle  |        |    |    |    |    |
| C-22       | 0.54 ± 0.33 | 0.31 ± 0.06 | 0.58 ± 0.08 | 1.0 ± 0.14 | 1.0 ± 0.14 |
| V-22       | 0.86 ± 0.23 | 1.3 ± 0.17 | 1.0 ± 0.27 | 1.6 ± 0.32 | 1.6 ± 0.32 |
| 3' Circle  |        |    |    |    |    |
| C-8        | 2.7 ± 0.23 | 0.5 ± 0.19 | 2.9 ± 0.20 | 1.7 ± 0.28 | 1.7 ± 0.28 |
| C-12       | 6.1 ± 0.68 | 0.16 ± 0.14 | 6.0 ± 0.52 | 0.60 ± 0.13 | 0.60 ± 0.13 |
| C-16       | 8.4 ± 0.13 | 0.16 ± 0.05 | 9.8 ± 0.31 | 0.96 ± 0.36 | 0.96 ± 0.36 |
| V-16       | 5.6 ± 0.24 | 0.60 ± 0.10 | 4.6 ± 0.17 | 0.37 ± 0.16 | 0.37 ± 0.16 |
| C-22       | 2.9 ± 0.73 | 0.67 ± 0.41 | 3.0 ± 0.31 | 1.1 ± 0.06 | 1.1 ± 0.06 |
| V-22       | 5.4 ± 0.37 | 0.53 ± 0.01 | 5.3 ± 0.56 | 0.97 ± 0.04 | 0.97 ± 0.04 |
| C-27       | 9.4 ± 0.69 | 0.89 ± 0.62 | 9.7 ± 0.30 | 1.4 ± 0.45 | 1.4 ± 0.45 |
| V-27       | 5.4 ± 0.25 | 1.1 ± 0.21 | 6.0 ± 0.50 | 2.2 ± 0.18 | 2.2 ± 0.18 |
| C-45       | 5.7 ± 0.70 | 0.72 ± 0.12 | 6.2 ± 0.33 | 3.3 ± 0.72 | 3.3 ± 0.72 |
| V-45       | 4.8 ± 0.12 | 0.67 ± 0.46 | 4.8 ± 0.31 | 4.1 ± 0.23 | 4.1 ± 0.23 |
| V-429      | 6.1 ± 1.0 | 1.7 ± 0.38 | 7.6 ± 0.35 | 2.7 ± 0.38 | 2.7 ± 0.38 |
| G-T        | 0.99 ± 0.30 | 0.09 ± 0.15 | 1.7 ± 0.15 | 5.2 ± 0.31 | 5.2 ± 0.31 |
| 5' Circle  |        |    |    |    |    |
| C-8        | 5.5 ± 0.25 | 0.73 ± 0.17 | 6.5 ± 0.79 | 1.0 ± 0.96 | 1.0 ± 0.96 |
| C-12       | 6.5 ± 1.1 | 0.27 ± 0.24 | 6.6 ± 0.48 | 0.67 ± 0.15 | 0.67 ± 0.15 |
| C-16       | 3.4 ± 0.2 | 0.38 ± 0.24 | 3.4 ± 0.23 | 0.51 ± 0.14 | 0.51 ± 0.14 |
| V-16       | 3.4 ± 0.4 | 0.46 ± 0.20 | 4.2 ± 0.26 | 0.75 ± 0.25 | 0.75 ± 0.25 |
| C-22       | 3.6 ± 0.33 | 0.24 ± 0.15 | 4.0 ± 0.25 | 0.90 ± 0.06 | 0.90 ± 0.06 |
| V-22       | 6.3 ± 0.60 | 0.41 ± 0.36 | 7.2 ± 0.63 | 1.0 ± 0.31 | 1.0 ± 0.31 |
| V-27       | 3.2 ± 0.54 | 0.90 ± 0.26 | 3.2 ± 0.25 | 1.5 ± 0.16 | 1.5 ± 0.16 |
| C-45       | 3.3 ± 0.12 | 0.77 ± 0.12 | 3.9 ± 0.25 | 1.5 ± 0.21 | 1.5 ± 0.21 |
| V-45       | 3.1 ± 0.12 | 1.1 ± 0.08 | 3.4 ± 0.12 | 2.5 ± 0.13 | 2.5 ± 0.13 |
| V-429      | 6.5 ± 0.50 | 1.7 ± 0.50 | 7.8 ± 0.60 | 2.5 ± 0.26 | 2.5 ± 0.26 |
| G-T        | 3.6 ± 0.56 | 0.48 ± 0.17 | 4.4 ± 0.35 | 3.5 ± 0.44 | 3.5 ± 0.44 |
Heteroduplex repair was determined as described under "Experimental Procedures," and the reaction (10 µl) contained mismatch repair-proficient (NM522) or -deficient (RK1517) extracts and 21 fmol of fully methylated circular heteroduplex DNA with the indicated heterology and nick position. Activity is expressed as repair occurring on either the closed viral strand (V+) or the nicked complementary strand (C+). Repair shown for each DNA strand is the average of at least three measurements ± S.D.

### Table III

**Methylation and nick dependence in loop repair**

| Substrates    | Repair C+ | Repair V+ | Repair C+ | Repair V+ |
|---------------|-----------|-----------|-----------|-----------|
| 3'-Circle     |           |           |           |           |
| G-T           | 1.0 ± 0.17| 0.21 ± 0.19| 1.1 ± 0.33| 0.33 ± 0.15|
| C45           | 4.4 ± 0.25| 0.48 ± 0.17| 3.8 ± 0.59| 1.0 ± 0.10|
| V45           | 5.2 ± 0.29| 1.1 ± 0.08| 3.9 ± 0.48| 0.70 ± 0.24|
| V429          | 6.1 ± 0.34| 2.9 ± 0.48| 5.8 ± 0.25| 2.4 ± 0.18|
| 5'-Circle     |           |           |           |           |
| G-T           | 6.2 ± 0.27| 1.1 ± 0.03| 5.5 ± 0.34| 0.61 ± 0.34|
| C45           | 3.0 ± 0.30| 1.2 ± 0.2 | 2.9 ± 0.25| 0.56 ± 0.08|
| V45           | 3.9 ± 0.40| 0.94 ± 0.26| 2.1 ± 0.19| 0.53 ± 0.17|
| V429          | 6.5 ± 0.60| 2.4 ± 0.37| 6.4 ± 0.17| 2.2 ± 0.42|

**Table IV**

**Reaction requirements of nick-directed loop repair**

| Reaction conditions | 3'-C16 | 3'-C27 | 3'-C45 | 3'-V429 |
|---------------------|--------|--------|--------|--------|
| Complete            | 100    | 100    | 100    | 100    |
| Without Mg          | <1     | <1     | <1     | <1     |
| Without ATP         | 100    | 98     | 117    | 115    |
| Without dNTPs       | 22     | 33     | 38     | 39     |

The major finding of this study is that repair of loops in *E. coli* extracts can be very efficient. *In vitro* assays with loops of 8, 12, 16, 22, 27, 45, and 429 nucleotides show a high level of correction for all substrates. Under these assay conditions, the efficiency of loop repair was comparable with that of methyl-directed mismatch repair. This extent of correction indicates that bacteria have substantial capacity to correct loop size well beyond the range of mismatch repair (14). The other significant conclusion from this study is that *in vitro* loop repair in bacteria shows close similarity to loop repair activities identified in extracts of yeast (20) and mammalian cells (16, 24, 26). The similarities of loop repair in prokaryotes and eukaryotes (20, 26) and the independence these systems with respect to mismatch repair components suggest conservation of function, although the activities responsible for large heterology repair have not been established in any organism.

Large heterologies were subject to limited processing when present in covalently closed circular heteroduplexes, but repair was enhanced substantially by a strand break placed 3' or 5' to the loop, and in this case, rectification was highly biased to the incised strand. This dependence on nick but not Dam methyltransferase was also distinguished by the reaction from mismatch repair, which can be directed to the unmethylated strand by a hemimethylated d(GATC) site. The two pathways are also different, as judged by the nature of reaction requirement (Table IV).

Transformation of *E. coli* cells with plasmid heteroduplexes by Carraway and Marinus (13) has previously indicated that large loops are not rectified in *E. coli* cells. In contrast, we have observed variable preference for large heterology separation distance.

Thus, although basal processing of unpaired heterologies does occur (Table II and Fig. 3, ccc entries), the presence of a strand break substantially increases the efficiency of the reaction and confers strand specificity on the process. The low levels of strand-independent reaction that we observe with covalently closed circular heteroduplexes could be the consequence of events directed by a strand break produced by endogenous endonucleases present in the extract. The same activity may produce the low level of nick-independent repair on the closed strand of the mutS reaction (Table II). However, with increasing length of heterology, we have occasionally observed higher levels of reaction on heteroduplexes that are independent of MutS and strand break (Table II, V- entries of V27, C45, V45, and V429 in RK1517). Consequently, it is possible that large loops may be directly recognized and processed in a nick-independent fashion by other activities. Repair in these cases may be mediated by mechanisms such as the pathway described by Fishel et al. (32).

**DISCUSSION**

The major finding of this study is that repair of loops in *E. coli* extracts can be very efficient. *In vitro* assays with loops of 8, 12, 16, 22, 27, 45, and 429 nucleotides show a high level of correction for all substrates. Under these assay conditions, the efficiency of loop repair was comparable with that of methyl-directed mismatch repair. This extent of correction indicates that bacteria have substantial capacity to correct loop size well beyond the range of mismatch repair (14). The other significant
DNA Loop Repair in E. coli

Mismatch repair-deficient cell extracts are capable of in vitro large loop repair in nick-directed fashion

Heteroduplex repair was determined as described under “Experimental Procedures,” and the reaction (10 μl) contained 75 μg of the indicated cell extracts and 21 fmol of circular heteroduplex DNA with the indicated heterology. C+, repair occurring on the nicked complementary strand; V−, repair occurring on the closed viral strand. Repair shown for each DNA strand is the average of at least three measurements ± S.D.

Table V

| Substrates | GM2931 (mutL) | GM3773 (mutH) |
|------------|---------------|---------------|
|            | C+            | V−            | C+           | V−           |
| 3′-Circle  |               |               |              |              |
| G-T        | 0.66 ± 0.21   | 0.52 ± 0.16   | 2.1 ± 0.44   | 1.5 ± 0.14   |
| C12        | 5.4 ± 0.41    | 0.27 ± 0.11   | 6.2 ± 0.19   | 0.37 ± 0.10  |
| V22        | 4.9 ± 0.65    | 0.41 ± 0.12   | 5.4 ± 0.16   | 0.53 ± 0.10  |
| C45        | 5.9 ± 0.32    | 1.2 ± 0.25    | 6.1 ± 0.51   | 1.5 ± 0.37   |
| V429       | 6.0 ± 0.31    | 1.6 ± 0.40    | 6.5 ± 0.25   | 2.4 ± 0.31   |
| 5′-Circle  |               |               |              |              |
| G-T        | 6.0 ± 0.07    | 1.4 ± 0.28    | 6.1 ± 0.64   | 0.68 ± 0.05  |
| C12        | 6.0 ± 0.15    | 0.13 ± 0.13   | 6.2 ± 0.17   | 0.35 ± 0.22  |
| V22        | 7.0 ± 0.50    | 0.47 ± 0.27   | 7.4 ± 0.20   | 0.68 ± 0.36  |
| C45        | 4.0 ± 0.33    | 0.63 ± 0.03   | 3.6 ± 0.57   | 0.45 ± 0.11  |
| V429       | 6.8 ± 0.30    | 1.6 ± 0.30    | 6.9 ± 0.30   | 2.2 ± 0.32   |

Fig. 3. Loop repair efficiency and nick positions. Heteroduplex repair of the C DNA strand was determined as described under “Experimental Procedures,” and the reaction (10 μl) contained 75 μg of NM522 cell extracts and 21 fmol of circular heteroduplex DNA with the indicated heterology. Heteroduplexes contained a single-strand break on the C strand at the EcoRI site 49 nucleotides 3′ to the heterology (3′-49), at the BglII site 150 nucleotides 5′ to the heterology (5′-150), at the BglII site 655 nucleotides 5′ to the heterology (5′-655), or at the AulNI site 3154 nucleotides 5′ to the heterology (5′-3154). Covalently closed circular (ccc) heteroduplexes were also tested. Heteroduplexes of C22 (black bar), V22 (white bar), and C45 (gray bar) were tested in this experiment. The error bars represent one S.D. from three determinations.

dh therefore seems highly unlikely that the correction events described above are a mere consequence of nonspecific nick translation. Effective correction of 3′-circles in E. coli cells also suggests that bacterial nick-directed loop repair has broader substrate specificity than that observed in the human pathway (26).

The demonstration of strand-specific repair of loops in E. coli extracts raises questions concerning the function of this reaction. Although such a system may function in the processing of recombination heteroduplexes, the strand specificity of the reaction suggests a role in correction of insertion/deletion heterologies that arise by DNA misalignment events during replication (1, 33). The observation of Fig. 3 that the efficiency of E. coli loop repair is independent of the distance separating the loop and the strand break suggests that the nick may act as a strand signal and not as a free end for excision and resynthesis. A nick as a strand signal and activator in heteroduplex corrections is not without precedent. Varlet et al. (34) demonstrated that DNA strand breaks act as signals rather than excision points in Xenopus mismatch repair. Furthermore, the experiments on the mechanism of ligation strand replication by E. coli DNA polymerase III holoenzyme demonstrated that the repli-

case cycles from one DNA site to another via preassembled DNA sliding clamps. It was suggested that the clamp, left on the DNA at the internal DNA termini (e.g. of the Okazaki fragments) may be harnessed by other machineries coordinated with chromosome replication (e.g. the repair and recombination systems) and used as a signal for the newly synthesized strand (35, 36). Therefore, editing of DNA replication and recombination processes by the loop repair components could be accomplished using strand discontinuities as strand discrimination signals.

Together, mismatch repair and loop repair provide complementary correction of loops that range from one to hundreds of nucleotides. There appears to be overlap between pathways for certain loops. Since we have found that repair of 45- and 429-nucleotide loops are partially dependent on MutS and methylation state, we propose that the sequence of the loop may adopt a configuration suitable for MutS recognition. These results imply that mismatch repair has, under certain circumstances, some activity on loops as large as 429 bases. The overlapping substrate specificities of mismatch and loop repair systems may serve to ensure coverage of a wide spectrum of possible insertion/deletion heterologies. Isolation of the activities involved in the pathway and the identification of the corresponding structural genes should serve to further clarify the roles of this system.

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DNA Loop Repair by *Escherichia coli* Cell Extracts

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