Redundant Exonuclease Involvement in Escherichia coli Methyl-directed Mismatch Repair*

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Previous biochemical analysis of Escherichia coli methyl-directed mismatch repair implicates three redundant single-strand DNA-specific exoenzymes (RecJ, ExoI, and ExoVII) and at least one additional unknown exoenzyme in the excision reaction (Cooper, D. L., Lahue, R. S., and Modrich, P. (1993) J. Biol. Chem. 268, 11823–11829). We show here that ExoX also participates in methyl-directed mismatch repair. Analysis of the reaction with crude extracts and purified components demonstrated that ExoX can mediate repair directed from a strand signal 3′ of a mismatch. Whereas extracts of all possible single, double, and triple exoexonuclease mutants displayed significant residual mismatch repair, extracts deficient in RecJ, ExoI, ExoVII, and ExoX exonucleases were devoid of normal repair activity. The RecJ− ExoVII− ExoX− strain displayed a 7-fold increase in mutation rate, a significant increase, but less than that observed for other blocks of the mismatch repair pathway. This elevation is epistatic to deficiency for MutS, suggesting an effect via the mismatch repair pathway. Our other work (Burdett, V., Baitinger, C., Viswanathan, M., Lovett, S. T., and Modrich, P. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6765–6770) suggests that mutants are under-recovered in the exonuclease-deficient strain due to loss of viability that is triggered by mismatched base pairs in this genetic background. The availability of any one exonuclease is enough to support full mismatch correction, as evident from the normal mutation rates of all triple mutants. Because three of these exoenzymes possess a strict polarity of digestion, this suggests that mismatch repair can occur exclusively from a 3′ or a 5′ direction to the mismatch, if necessary.

The correction of DNA polymerase misincorporation errors plays an important role in the maintenance of genetic integrity. DNA synthesis errors in Escherichia coli are corrected by the methyl-directed mismatch repair (MMR)1 system, which removes incorrect nucleotides by a strand-specific excision reaction that is directed to daughter DNA strands by virtue of the transient absence of d(GATC) methylation within newly synthesized DNA (1). This system processes both base-base and small insertion/deletion mismatches (2–4). Inactivation of the genes encoding the nonessential MutH, MutL, MutS, or UvrD components confers a large increase (100–500-fold) in mutation rate (1, 5).

Biochemical analysis of the repair of artificial mismatch-containing substrates has defined 10 activities in a methyl-directed excision repair reaction reconstituted with purified components (6, 7). Repair is initiated by the binding of MutS to the mismatch (2, 3, 8), with MutL binding to the heteroduplex in a MutS- and ATP-dependent manner (9–11). Assembly of this ternary complex is sufficient to activate the d(GATC)endonuclease activity of MutH, which incises the unmethylated strand of a hemimethylated d(GATC) sequence (12), as well as the unwinding activity of DNA helicase II (uvrD/mutU gene product), which enters the helix at the incised d(GATC) sequence and unwinds toward the mismatch (13). That portion of the unwound, incised strand is subject to degradation by one of several single-strand DNA (ssDNA) exonucleases (7, 14), and DNA removed in this manner is resynthesized by DNA polymerase III holoenzyme in the presence of single-strand DNA-binding protein. Finally, DNA ligase restores covalent continuity to the repaired strand (6).

In vitro excision can be directed by an incised d(GATC) site that is located either 5′ or 3′ to the mismatch on the unmethylated strand, and the nature of the exonuclease requirement depends on the relative orientation of the two DNA sites (7). If the initial incision is made 5′ to the mismatch, excision requires either ExoVII or RecJ exonuclease, both of which are capable of 5′→3′ hydrolysis of ssDNA (15, 16), whereas exonuclease I, which is capable of 3′→5′ hydrolytic activity (17), is sufficient to meet the exonuclease requirement when d(GATC) incision occurs 3′ to the mispair. Extracts prepared from a RecJ− ExoVII− double mutant are defective in repair directed by a 5′ d(GATC) sequence. However, extracts of ExoI-deficient strains retain repair activity on heteroduplexes where repair is directed by a d(GATC) site located 3′ to the mismatch, suggesting that at least one additional activity can meet the 3′ exonuclease requirement (7).

The phenotypes of strains mutant in RecJ, ExoI, and/or ExoVII exonucleases have implicated these genes and their products in DNA repair and recombination (18–20). In addition, ExoI and ExoVII also appear to have overlapping roles in preventing frameshift and quasi-palindrome templated mutations, two classes of mutations associated with strand slippage during DNA replication (21, 22). Despite the biochemical evidence implicating ExoI, RecJ, and ExoVII in mismatch repair (7), triple mutants deficient in the three activities fail to show...
enhanced mutability in assays that score for spontaneous base substitutions, a hallmark of MMR deficiency (21, 23). However, it seems unlikely that DNA helicase II alone would be sufficient to support methyl-directed excision, especially when one considers that excision tracts can be a thousand nucleotides or more (14, 24). Inasmuch as ExoI, RecJ, and ExoVII have been implicated in a UV-induced recombination pathway that also depends on MutH, MutL, and MutS mismatch repair activities (25), and because biochemical experiments have suggested involvement of at least one additional exonuclease in the methyl-directed reaction, we have sought the identity of other exonucleases that might support methyl-directed excision.

A novel E. coli 3’→5’ exonuclease, exonuclease X, has recently been identified (26). This activity hydrolyzes both single- and double-strand DNA, but its affinity for single-strand DNA is approximately 10 times greater than that for duplex DNA. Unlike RecJ, ExoI, and ExoVII, which are proccessive exonucleases, ExoX is distributive, hydrolyzing only one or a few nucleotides before releasing from its substrate. The fact that ExoX is distributive does not affect its potency as a nuclease; in fact, its affinity for single-strand DNA and its specific activity rival those of RecJ and ExoI.

We demonstrate here that ExoX also supports methyl-directed mismatch repair in vitro. The results show that either RecJ or ExoVII is sufficient to meet the exonuclease requirement when excision is initiated 5’ to the mispair, whereas either ExoI, ExoVII, or ExoX is sufficient to meet the exonuclease requirement when excision is initiated 3’ to the mismatch. Simultaneous inactivation of ExoX, Exol, RecJ, and ExoVII abolishes normal mismatch repair in vitro but confess only a modest increase in mutation rate. We provide evidence elsewhere (27) that these exonucleases participate in methyl-directed repair in vivo, and we demonstrate that the relatively low mutability of the quadruple exonuclease mutant is due to under-recovery of mutants as a consequence of lethal events triggered by the occurrence of mismatches in this genetic background.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Media, and Antibiotics—Isogenic strains listed in Table I, which were derived from BT199, were constructed by P1 phage-mediated transduction (28). Strains were grown in LB medium (1% tryptone, 0.5% yeast extract, and 1% NaCl) or on solid LB medium containing 1.4% agar. Medium for P1 lysates and transductions consisted of LB medium supplemented with 10 mM calcium chloride and 0.2% glucose. Transductions were selected on LB agar containing 10 mM sodium citrate supplemented with ampicillin, tetracycline, kanamycin, or chloramphenicol at concentrations of 100, 15, 25, and 15 μg/ml, respectively. LB media for mutation assays contained rifampicin (Rif; 100 μg/ml). Growth was at 37 °C, unless otherwise indicated.

Mutation Assays—Mutation assay scoring for Rif resistance was performed as described previously (21). Mutation rates were calculated by the method of the median (29) using the following formula: mutation rate = MIN, where M is the calculated number of mutation events, and N is the mean number of viable cells in the culture. M is solved by interpolation from experimental determination of re, the median number of mutant cells determined among the cultures for each set of assays, by the formula re = M(1.24 + lnM). The 95% confidence intervals were calculated as described previously (30). For experiments involving the temperature-sensitive allele of recJ, culture growth and selection for Rif-resistant colonies were maintained at 30 °C or 42 °C, as indicated.

In Vitro Mismatch Repair Assays—E. coli extracts were prepared as described previously (7). Briefly, ammonium sulfate pellets were resuspended in 25 mM HEPES (potassium salt, pH 7.6), 0.1 mM EDTA, 2 mM dithiothreitol, and 50 mM KCl and dialyzed against this buffer until the conductivity of the sample was equivalent to that of 25 mM HEPES buffer containing 150 mM KCl. Mismatch repair in cell-free extracts was performed at 37 °C in 20 mM Tris-HCl buffered reactions (pH 7.6) containing 90 mM KCl (final concentration including the contribution from the extract) as described previously (7), except that reactions (20 μl) contained 24 fmol of heteroduplex and ~0.1 mg of extract protein. When indicated, reactions were performed in a 20 mM Tris-HCl buffer (pH 8) with a reduced salt concentration (55 mM KCl).

Exonuclease I (U.S. Biochemical Corp.) used for extract complementation was dialyzed against 20 mM Tris-HCl (pH 7.6), 150 mM KCl, 0.5 mM EDTA, and 5 mM 2-mercaptoethanol for 6 h at 4 °C (with two buffer changes) to remove glycerol, a potent inhibitor of the reaction in vitro. The dialyzed extract was diluted to 10 μg/ml in 20 mM KPO4 (pH 7.4), 50 mM KCl, 1 mM EDTA, 2 mM dithiothreitol, 100 μg/ml bovine serum albumin, and 1 μl was added to repair reactions.

Repair in a purified system was performed at 37 °C, essentially as described previously (6, 7). Reactions contained (per 20 μl) 24 fmol of heteroduplex DNA, 35 ng of MutS, 30 ng of MutL, 0.26 ng of MutH, 3 ng of DNA helicase II, 150 ng of single-stranded DNA-binding protein (SSB), 20 ng of E. coli DNA ligase, 100 ng of DNA polymerase III holoenzyme (generously provided by Dr. Mike O'Donnell, Rockefeller University), and exonuclease(s) as indicated.

RESULTS

E. coli Deficient in ExoI, ExoVII, ExoX, and RecJ Exonucleases Display a Modest Mutator Phenotype—Previous work implicated three ssDNA-specific exonucleases (ExoI, RecJ, and ExoVII) in methyl-directed mismatch repair in vitro and indicated that at least one additional activity also participates in the pathway (7). To examine the possible role of exonuclease X in this regard, an isogenic set of mutant strains was constructed with null alleles of ExoX and the three ssDNA-specific exonucleases that are known to participate in mismatch correction (Table I). This panel of mutants constituted a set of strains defective in all possible combinations of one, two, three, or four null alleles of the single-strand exonucleases cited above. The rate of spontaneous mutation was determined for these strains in an assay for Rif resistance, which scores for base substitution errors. The ExoX single mutant as well as all triple exonuclease mutant combinations (Table II) showed no significant increase in mutation rate compared with the ExoX control strain. The quadruple mutant deficient in all four exonucleases exhibited a moderate increase (7-fold) in mutation rate, suggesting a potential defect in DNA repair. A quadruple exonuclease mutant with a temperature-sensitive allele of recJ, recJ146 (20), exhibited a temperature-dependent increase in mutation rate (Table III), confirming that it is exonuclease deficiency that is responsible for the mutator phenotype. The elevation of mutation rate in the quadruple null exonuclease mutant is considerably less than that in strains defective in MutS or UvrD, key components of the methyl-directed repair system of E. coli, which exhibited a 30–100-fold increase in mutation rate relative to the isogenic wild type control (Table II). The mutation rates in MutS+ or UvrD+ derivatives of the ExoVII RecJ ExoX ExoVII strain approximated those observed with the MutS+ or UvrD+ strains, suggesting that the bulk of the mutator effect associated with exonuclease deficiency is caused by an inefficient mismatch repair pathway.

Orientation-dependent Exonuclease Requirements for In Vitro Mismatch Repair—Circular G-T heteroduplexes containing a single asymmetricaly located, hemimethylated d(GATC) site have been used previously to study the exonucleases involved in the excision step of methyl-directed mismatch repair in vitro (7, 14). DNAs of the type shown in Fig. I have been used to study the mismatch-dependent excision reaction that removes the portion of the unmethylated strand spanning the shorter path between the two DNA sites irrespective of placement of the unmethylated d(GATC) sequence 3′ or 5′ to the mispair. Using such 3′ and 5′ heteroduplexes, we have examined mismatch repair in cell-free extracts derived from wild type E. coli and an otherwise isogenic quadruple mutant deficient in ExoVII, RecJ, ExoI, and ExoX, as well as in all possible single, double, and triple mutants (Table IV).

Extracts of wild type BT199 support efficient repair of both 5′ and 3′ heteroduplexes. As shown in Table IV, deficiency of a
single exonuclease has only a modest effect on repair rates. Genetic inactivation of both ExoVII and RecJ (STL4556) abolished repair on the 5° heteroduplex, confirming a previous observation (7). Both RecJ and ExoVII have 5°–3° exonuclease activity (15, 16), and both have been previously shown to be functionally redundant for 5° heteroduplex repair (7). Deficiency of ExoI (STL2694), ExoX (STL4534), or both 3°–5° hydrolytic activities (STL4538) was without effect on repair of the 5° G-T heteroduplex. ExoI deficiency (STL2694) reduced correction of the 3° heteroduplex by 50%, and deficiency of both ExoI and ExoX reduced repair by 63% (STL4538). Residual 3° repair in this double mutant is attributable to the 3°–5° activ-

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**TABLE I**

**E. coli K-12 strains**

| Strain   | Genotype | Source or derivation |
|----------|----------|----------------------|
| BT199    | F- θ thi-1 Δcmt-proA62 thr-l lacB168 hfdK51 rfdD1 ara-14 lacY1 galK2 xyl-5 mtl-1 supE44 rpsL31 strC | W. Wackernagel |
| GM4799   | + mutS488::minT10kan argE3 hisG4       | M. Marinus         |
| JCl2170  | + recJ14G(s) recB21 recC22 sbeB15 sbeC201 argE3 hisG4 | Ref. 20           |
| RDK1445  | + serA6 zge-224::Tn10 argE3 hisG4       | R. Kolodner        |
| STL1536  | + uvrD24::Tn5 argE3 hisG4              | Ref. 37           |
| STL2351  | + recA284::Tn10                      | Ref. 21           |
| STL2694  | + Δexon300::cat                      | Ref. 21           |
| STL2729  | + recJ284::Tn10 Δexon300::cat         | Ref. 21           |
| STL4150  | + Δexon300::cat xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4525  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4544  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4537  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4538  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4539  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4540  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4541  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4542  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4543  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4545  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4555  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4556  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL4557  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL5815  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL5871  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL6013  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| STL6014  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| VB1-VB4  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| SMR3472  | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| S17-1pir | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |
| S17-1pir | + Δexon11::npt xseA18::amp recJ284::Tn10 | Ap” transductant SMR3472 X STL2729 |

**TABLE II**

**Mutation rate to rifampicin resistance for exonuclease-deficient strains**

| Strain   | Description | n | Rif" mutation rate × 10⁹ (95% confidence interval) | Relative mutation rate |
|----------|-------------|---|-----------------------------------------------|-----------------------|
| BT199    | Wild-type   | 52 | 3.6 (1.8–4.6) | 1                     |
| STL4534  | ExoX        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| GM4799   | MutS-       | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL1526  | UvrD        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL4150  | RecJ        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL4539  | RecJ        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL4542  | RecJ        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL4541  | RecJ        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| VB1      | RecJ        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL6014  | ExoX        | 28 | 3.0 (1.5–5.0) | 0.8                  |
| STL6013  | ExoX        | 28 | 3.0 (1.5–5.0) | 0.8                  |

**TABLE III**

**Temperature sensitivity of exonuclease-associated mutator phenotype**

The temperature-sensitive allele recJ146 (20) was used in these experiments. VB1 could not be assayed at 30 °C because the strain is cold-sensitive for growth.

| Strain   | Description | T °C | n | Rif" mutation rate × 10⁹ (95% confidence interval) |
|----------|-------------|-----|---|-----------------------------------------------|
| STL4541  | ExoX        | 30  | 16 | 3.0 (1.8–7.0) | |
| STL5871  | RecJ        | 30  | 33 | 3.0 (1.8–7.0) | |
| STL4541  | ExoX        | 42  | 64 | 3.5 (1.8–4.6) | |
| STL5871  | RecJ        | 42  | 40 | 7.5 (6.2–9.0) | |
| VB1      | RecJ        | 42  | 70 | 16 (13–16)   | |

* a, cell growth and assay temperature.  
* b, number of independent cultures assayed.  
* c, temperature-sensitive.
ity of ExoVII (15) because introduction of an ExoVII null mutation into the ExoI ExoX abounded background abolished repair activity on the 3' heteroduplex (STL4541). These findings indicate that either ExoVII or RecJ is sufficient to meet the exonuclease requirement for repair of 5' heteroduplexes in E. coli extracts, whereas ExoI, ExoX, or ExoVII can each contribute in a redundant fashion to repair of 3' heteroduplexes.

With one exception, the repair efficiencies of triple mutants that retain only one of the four activities were consistent with these polarity assignments. Thus, STL4542 (ExoVII RecJ ExoX ExoI) supported efficient repair (57% of wild type) of the 3' heteroduplex but displayed only trace activity on the 5' substrate, STL4541 (ExoVII RecJ ExoX ExoX) displayed about 50% of wild type repair activity on the 5' heteroduplex but was inactive on the 3' substrate, and STL4539 (ExoVII RecJ ExoI ExoX) supported significant repair with both heteroduplex orientations. Surprisingly, extracts of STL4150 (ExoVII RecJ ExoI ExoX) supported rectification of both 3' (15% of wild type) and 5' (80% of wild type) heteroduplexes, despite the fact that ExoX is known to hydrolyze DNA with strict 3'→5' polarity, and extracts of STL4556 (ExoVII RecJ ExoI ExoX) are devoid of repair activity on the 5' heteroduplex. We also noted that whereas STL4150 extracts displayed about 15% of the wild type level of activity on the 3' heteroduplex, activity was increased to 30% of the wild type level by a slight change in assay conditions (Table III, parenthetic values; see "Experimental Procedures"). Extracts of VB2 (ExoVII RecJ ExoI ExoX) showed less than 2% of the wild type levels of repair of 3' substrate under the standard assay conditions; however, like STL4150, these extracts also supported apparent repair of 5' heteroduplex (60% of wild type). Repair of both 3' and 5' heteroduplexes in extracts of STL4150 (ExoVII RecJ ExoI ExoX) and VB1-VB4 (ExoVII RecJ ExoI ExoX) was strand-specific (data not shown).

The nature of the unanticipated 5' repair activity in STL4150 was addressed in several ways. Introduction of a wild type ExoI allele into STL4150 yielded a strain with an in vitro repair specificity identical to that of STL4556 (ExoVII RecJ ExoI ExoX) (data not shown), implying that the 5' repair observed in STL4150 extracts is not a consequence of genetic activation of a known 5'→3' exonuclease in this genetic background. Indeed, as shown in Figs. 2 and 3, the 5' heteroduplex repair observed in STL4150 (ExoVII RecJ ExoI ExoX) and VB1-VB4 (ExoVII RecJ ExoI ExoX) extracts was suppressed upon mixing with extract derived from STL4542 (ExoVII RecJ ExoI ExoX) or upon the addition of purified exonuclease I, although in both cases 3' heteroduplex repair was enhanced as expected. These results mimic the repair activities obtained with STL4556 (ExoVII RecJ ExoI ExoX). Furthermore, as shown below, ExoX supports repair of only the 3' heteroduplex in the reconstituted mismatch repair system. We therefore attribute the apparent repair of the 5' heteroduplex in STL4150 extracts to molecular events that are suppressed in the presence of ExoI, despite the strict 3'→5' hydrolytic polarity of this activity (17).

The nature of the anomalous 5' heteroduplex rectification observed in STL4150 (ExoVII RecJ ExoI ExoX) and VB2 (ExoVII RecJ ExoI ExoX) extracts is unclear. As described below, a related phenomenon was also observed in the purified system. As shown in Fig. 1, in vitro repair is scored by cleavage with a restriction enzyme diagnostic for correction as well as Bsp106I. Repaired molecules thus yield two fragments of 3.1 and 3.3 kbp that are recovered with 1:1 molar stoichiometry. Signifigant deviation from molar equivalence of these two species was observed for repair of the 5' substrate in STL4150 (ExoVII RecJ ExoI ExoX) and VB1-VB4 (ExoVII RecJ ExoI ExoX) extracts, with the smaller 3.1-kbp product consistently under-represented, indicating that these repair events are to some degree anomalous. The ability of ExoI to suppress these events in extracts raises the possibility of preferential interaction between this activity and other components of the mismatch repair system. The possibility of interaction of exonucleases with each other or with other components of the repair system is also suggested by comparison of the levels of 3' heteroduplex repair observed in STL4555 (ExoVII RecJ ExoI ExoX) and STL2729 (ExoVII RecJ ExoI ExoX) with that observed in STL4150 (ExoVII RecJ ExoI ExoX). The level of 3' heteroduplex repair activity observed in extracts containing both RecJ and ExoX or both ExoVII and ExoX was significantly less than that observed with STL4150 extracts that contain only ExoX; moreover, in contrast to 3' repair observed in STL4150 extracts, the activity in the former strains failed to respond to alteration of reaction conditions (Table IV).

Reconstituted Mismatch Repair—ExoVII and RecJ have been previously shown to be required for 5' heteroduplex repair in a purified system, whereas ExoI was implicated in correction of 3' heteroduplexes (7). The repair specificity of ExoX has now been assessed in the reconstituted system. As shown in Fig. 4, ExoX supports repair of the 3' heteroduplex in the purified system (lane 4) but displays little if any activity on the 5' heteroduplex (lane 8). Previous work with the purified system suggested that the action of ExoVII was restricted to repair of 5' heteroduplexes (7). However, the extract experiments described above indicated that ExoVII also contributes to rectification of 3' heteroduplexes. Consequently, we have re-examined this question in the purified system. As shown in Fig. 4 (lane 7), ExoVII is highly active in 5' heteroduplex correction, but this activity also supports a low but significant level of repair on the 3' heteroduplex substrate (lane 3). These results are consistent with the extract results described above. In the absence of 5' exonucleases, a striking feature of the reactions involving the 5' substrate is the presence of an apparent repair band of 3.3 kbp, with under-representation or absence of the expected 3.1-kbp repair band and the appearance of anomalous species between the 3.3-kbp species and full-length linear heteroduplex. Reconstitution experiments (data not shown) demonstrated that this effect is dependent on MutS, MutL, MutH, helicase II, SSB, and polymerase III holoenzyme. We think it likely that these anomalous repair bands are due to helicase II strand displacement from the MutH-incised d(GATC) site coupled with extension by polymerase III of the exposed terminus. We postulate that the anomalous 5' repair observed in extracts of STL4150 (ExoVII RecJ ExoI ExoX) or VB2 (ExoVII...
RecJ− ExoI− Exo−), discussed above, has a similar explanation in that there is no suitable exonuclease present that can degrade a displaced 5′ single strand.

**DISCUSSION**

Previous work has implicated *E. coli* ExoI, as well as the ssDNA exonucleases ExoI, ExoVII, and RecJ, in the repair of UV-damaged DNA (21, 26). We have now demonstrated that these ssDNA exonucleases also function redundantly during mismatch correction. Inactivation of ExoI, ExoVII, ExoX, and RecJ conferred a moderate mutator phenotype, and the corresponding cell-free extracts were devoid of normal mismatch repair activity in vitro on model heteroduplexes.

The modest increase in mutability observed in the absence of these four activities is less than that observed for other blocks of the MMR pathway, including mutS, mutL, mutH, or uvrD mutant strains. There are two possible explanations for this paradox. The simple heteroduplexes used for biochemical assay may not be good models for natural substrates. Consequently,

| Strain   | 5′→3′ + 3′→5′ ExoVII | 5′→3′ RecJ | 3′→5′ ExoI | 3′→3′ ExoX | 5′ Repaira | 3′ Repaira |
|----------|-----------------------|------------|------------|------------|------------|------------|
| BT199    | +                     | +          | +          | +          | 20 ± 12 (10.5) | 30 ± 9.8 (17 ± 2.4) |
| STL4534  | +                     | +          | +          | −          | 20 ± 5.3    | 26 ± 8.0    |
| STL2694  | +                     | +          | −          | +          | 24 ± 9.3    | 15 ± 2.5    |
| STL2331  | +                     | −          | +          | +          | 16 ± 5.0    | 35 ± 3.8    |
| STL4537  | −                     | +          | +          | +          | 18 ± 6.7    | 36 ± 2.0    |
| STL4556  | +                     | −          | +          | +          | <0.5 (1.9)  | 29 ± 11     |
| STL4538  | +                     | +          | −          | +          | 24 ± 5.4    | 11 ± 3.5    |
| STL4555  | −                     | +          | −          | +          | 17 ± 4.2 (14)| 1.3 ± 0.7 (1.2) |
| STL2729  | +                     | −          | −          | +          | 11 ± 3.4 (8.2)| 1.8 ± 0.7 (2.0)|
| STL4557  | −                     | +          | −          | +          | 3.4 ± 1.4   | 16 ± 3.1    |
| STL4540  | +                     | +          | +          | −          | 4.6 ± 1.8 (6.0)| 16 ± 1.7    |
| STL4150  | −                     | +          | −          | +          | 16 ± 1.6 (19 ± 12)| 4.7 ± 1.1 (11 ± 3.0)|
| STL4145  | −                     | −          | +          | +          | 1.8 ± 0.5 (2.9)| 17 ± 6.9    |
| STL4541  | −                     | −          | +          | −          | 11 ± 2.0 (12.6)| <0.5 (2.0) |
| STL4539  | +                     | −          | −          | +          | 6.4 ± 1.0 (8.6)| 3.3 ± 0.7 (2.1)|
| VB1–VB4  | −                     | −          | −          | +          | 11.1 ± 3.9 (15.3 ± 3.2)| 0.55 ± 0.44 (1.6 ± 0.6)|

Fig. 2. **Methyl-directed mismatch repair in vitro.** Mismatch repair assays were performed as described under “Experimental Procedures” for 45 min in reactions containing 150–180 µg of protein (for complementation, 75–90 µg of each extract was used). Repair products were scored by cleavage with Bsp10I6 and either XhoI (3′ heteroduplex) or HinIII (5′ heteroduplex) that yield 3.1- and 3.3-kbp products from repaired DNA (Fig. 1). The 3′ and 5′ heteroduplexes were identical except for the state of methylation of the single d(GATC) site: the 3′ substrate was methylated on the complementary strand, and the 5′ heteroduplex was methylated on the viral strand (2). Genotypes of strains used are summarized in Table I, and repair bands are indicated by arrows. Repair activity is expressed as fmol/h/0.1 mg protein.

| Strain   | 3′ Repair | 5′ Repair |
|----------|-----------|-----------|
| BT199 mutS | ++        | +         |
| STL4150  | +         | ++        |
| VB2      | +         | +         |
| STL4542  | +         | +         |

**TABLE IV**

In vitro repair with extracts from exonuclease-deficient strains

| Strain   | 5′→3′ + 3′→5′ ExoVII | 5′→3′ RecJ | 3′→5′ ExoI | 3′→3′ ExoX | 5′ Repair | 3′ Repair |
|----------|-----------------------|------------|------------|------------|------------|------------|
| BT199    | +                     | +          | +          | +          | 20 ± 12 (10.5) | 30 ± 9.8 (17 ± 2.4) |
| STL4534  | +                     | +          | +          | −          | 20 ± 5.3    | 26 ± 8.0    |
| STL2694  | +                     | +          | −          | +          | 24 ± 9.3    | 15 ± 2.5    |
| STL2331  | +                     | −          | +          | +          | 16 ± 5.0    | 35 ± 3.8    |
| STL4537  | −                     | +          | +          | +          | 18 ± 6.7    | 36 ± 2.0    |
| STL4556  | +                     | −          | +          | +          | <0.5 (1.9)  | 29 ± 11     |
| STL4538  | +                     | +          | −          | +          | 24 ± 5.4    | 11 ± 3.5    |
| STL4555  | −                     | +          | −          | +          | 17 ± 4.2 (14)| 1.3 ± 0.7 (1.2) |
| STL2729  | +                     | −          | −          | +          | 11 ± 3.4 (8.2)| 1.8 ± 0.7 (2.0)|
| STL4557  | −                     | +          | −          | +          | 3.4 ± 1.4   | 16 ± 3.1    |
| STL4540  | +                     | +          | +          | −          | 4.6 ± 1.8 (6.0)| 16 ± 1.7    |
| STL4150  | −                     | +          | −          | +          | 16 ± 1.6 (19 ± 12)| 4.7 ± 1.1 (11 ± 3.0)|
| STL4145  | −                     | −          | +          | +          | 1.8 ± 0.5 (2.9)| 17 ± 6.9    |
| STL4541  | −                     | −          | +          | −          | 11 ± 2.0 (12.6)| <0.5 (2.0) |
| STL4539  | +                     | −          | −          | +          | 6.4 ± 1.0 (8.6)| 3.3 ± 0.7 (2.1)|
| VB1–VB4  | −                     | −          | −          | +          | 11.1 ± 3.9 (15.3 ± 3.2)| 0.55 ± 0.44 (1.6 ± 0.6)|

Fig. 3. Exonuclease I restores 3′ repair and suppresses apparent 5′ repair in extracts of STL4150 (ExoI− ExoVII− RecJ− ExoX−) and VB2 (ExoVII− RecJ− ExoX−). Mismatch repair assays (“Experimental Procedures”) contained extract from STL4150 (lanes 1–4) or VB2 (lanes 5–8). Reactions corresponding to lanes 1, 2, 5, and 6 utilized 3′ heteroduplex, and those for lanes 3, 4, 7, and 8 contained 5′ heteroduplex. Reactions for odd-numbered lanes were un-supplemented, whereas reactions for even-numbered lanes were supplemented with 10 ng of exonuclease I.
the four exonucleases studied here may process some but not all mismatches in the cell. The alternate possibility is that all four exonucleases contribute to most, if not all, methyl-directed repair events—the relatively low mutability of the exonuclease-deficient strain may be due to under-recovery of mutants due to lethal events triggered by the occurrence of mismatches in this genetic background. We present evidence elsewhere (27) that the latter explanation is likely to be correct. Quadruplets mutS, mutL, mutH, or uvrD, genes that mediate the earlier steps in the MMR pathway. The exact nature of this lethality is not known, but presumably accumulation of ssDNA displaced during MMR is deleterious to the cell.

Depending on the relative position of the mistranscribed to the incision site, different exonucleases are recruited for the excision step of MMR. ExoI, ExoX, and ExoVII are all capable of participating in the 3’ repair pathway. Both ExoI and ExoX are strict 3’ to 5’ exonucleases (26, 32), and repair of the 3’ substrate in extracts deficient in these two exonucleases is reduced by 50%. No repair activity above background was detected in extracts when a null mutation in ExoVII was introduced into the ExoI–ExoX–deficient background. The results of this study differ from our earlier finding with respect to the ability of ExoI to support repair of a 3’ heteroduplex. Whereas previous work failed to detect 3’ heteroduplex repair supported by ExoVII in the reconstituted system (7), we now find that ExoVII can support 3’ heteroduplex repair in cell-free extracts and the purified system. However, in agreement with the previous study, we find that ExoVII is much less effective in supporting 3’ heteroduplex repair as compared with its activity with 5’ substrates (Fig. 4, compare lanes 3 and 7). In agreement with earlier results (7), we show here that RecJ or ExoVII is sufficient for removal of mismatched bases via excision tracts initiated 5’ at the mismatch. Thus both the 3’ and 5’ exonuclease activities of ExoVII can be utilized in the mismatch correction system.

These findings imply redundancy of exonuclease involvement in this mutation avoidance pathway and the level of repair supported by any one of these exonuclease activities is apparently sufficient to maintain a low level of spontaneous mutability. Mismatch repair proficiency is exhibited in the absence of all but one of these four exonucleases, further demonstrating that mismatch excision in vivo is truly bidirectional and can be accomplished from a single direction if necessary.

Although recJ and xae (ExoVII large subunit) orthologues can be found in almost all bacterial genomes sequenced to date, the other exonucleases are apparently more restricted in their distribution. This means that, at least in bacteria, a different assortment of exonucleases is likely to be called into play for the excision step of MMR in different species. The redundancy of exonuclease functions in E. coli may be due to the specialized roles these enzymes play in other aspects of DNA metabolism. For instance, RecJ mediates recombination via the RecF pathway (20) and may process stalled replication forks (33). RecJ and ExoI also assist recombination via the RecBCD pathway (21, 34–36). ExoI and ExoVII play important roles in the avoidance of misalignment errors during replication, such as frameshifts (21) and quasi-palindrome templated mutations (22).

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