Single-Strand DNA-Specific Exonucleases in *Escherichia coli*: Roles in Repair and Mutation Avoidance

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ABSTRACT

Mutations in the genes encoding single-strand DNA-specific exonucleases (ssExos) of *Escherichia coli* were examined for effects on mutation avoidance, UV repair, and conjugational recombination. Our results indicate complex and partially redundant roles for ssExos in these processes. Although biochemical experiments have implicated RecJ exonuclease, Exonuclease I (ExoI), and Exonuclease VII (ExoVII) in the methyl-directed mismatch repair pathway, the RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutant did not exhibit a mutator phenotype in several assays for base substitution mutations. If these exonucleases do participate in mismatch excision, other exonucleases in *E. coli* can compensate for their loss. Frameshift mutations, however, were stimulated in the RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutant. For acridine-induced frameshifts, this mutator effect was due to a synergistic effect of ExoI$^-$ and ExoVII$^-$ mutations, implicating both ExoI and ExoVII in avoidance of frameshift mutations. Although no single exonuclease mutant was especially sensitive to UV irradiation, the RecJ$^-$ ExoVII$^-$ double mutant was extremely sensitive. The addition of an ExoI$^-$ mutation augmented this sensitivity, suggesting that all three exonucleases play partially redundant roles in DNA repair. The ability to inherit genetic markers by conjugation was reduced modestly in the ExoI$^-$ RecJ$^-$ mutant, implying that the function of either ExoI or RecJ exonucleases enhances RecBCD-dependent homologous recombination.

IN the bacterium *Escherichia coli*, three exonucleases specifically degrade single-strand DNA (ssDNA): RecJ exonuclease (RecJ), Exonuclease I (ExoI), and Exonuclease VII (ExoVII). Each exonuclease has unique biochemical properties. RecJ degrades ssDNA with 5′ to 3′ polarity (Lovett and Kolodner 1989), ExoI has 3′ to 5′ polarity (Lehman and Nussbaum 1964), and ExoVII can degrade from either DNA end (Chase and Richardson 1974). Both RecJ (S. T. Lovett and R. D. Kolodner, unpublished results) and ExoI (Prasher *et al.* 1983) are monomeric proteins and require Mg^{2+} as a cofactor for activity; ExoVII is a heterodimer and does not require exogenous Mg^{2+} cofactor (Vales *et al.* 1982).

All three exonucleases have been implicated in the methyl-directed mismatch repair system (Cooper *et al.* 1993; Grilley *et al.* 1993) of *E. coli*. In concert with the mismatch recognition proteins MutS and MutL, the endonuclease MutH, and the DNA helicase UvrD, these exonucleases *in vitro* excise a DNA tract carrying a mispair. Either RecJ or ExoVII is required if the MutH-mediated incision is 5′ to the mismatched base. Purified ExoI can mediate excision if the incision is 3′ to the mismatch. However, unlike crude extracts from a RecJ$^-$ ExoVII$^-$ mutant that are defective in 5′ excision, ExoI$^-$ mutant extracts are proficient for excision 3′ to the mismatch (Cooper *et al.* 1993). This suggests the presence of another 3′ ssDNA exonuclease that may contribute to mismatch repair in *E. coli*. Despite the dual polarity of ExoVII, only the 5′ activity contributes to excision in this assay.

Although biochemical experiments suggest that the ssDNA-specific exonucleases (ssExos) can act during mismatch repair, mutations affecting all three exonucleases have not been tested for their effects on mutation avoidance. If these exonucleases are the only exonucleases that participate in methyl-directed mismatch repair, we would expect the triple RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutant to exhibit high levels of spontaneous mutations (a "mutator" phenotype). Moreover, other types of DNA repair requiring DNA excision may also be defective in ssExo-deficient strains.

We present here a genetic characterization of multiple mutants of the RecJ, ExoI, and ExoVII exonucleases. From normal spontaneous mutation rates for several assays of base substitutions, we conclude that either these exonucleases do not participate in methyl-directed mismatch repair or that additional unknown exonuclease activities can substitute fully for their loss. However, Exonucleases I and VII are apparently involved in avoidance of frameshift mutations by an unknown mechanism. All three exonucleases are required for repair of UV-induced damage, with RecJ and ExoVII playing a more prominent role. Either RecJ or ExoI is required for optimal recombination after conjugation. These complex properties suggest that ssDNA-specific exonucleases are

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both specialized and redundant for various aspects of DNA metabolism *in vivo*.

MATERIALS AND METHODS

Bacterial strains, media, and antibiotics: Isogenic strains, listed in Table 1, are derived from BT199 and were constructed by P1 transduction (Miller 1992). Strains were grown at 37° in Luria broth (LB) medium composed of 1% bacto-tryptone, 0.5% yeast extract, 0.5% NaCl; plate media contained 1.5% agar. Plate minimal medium consisted of 56/2 salts (Willetts *et al.* 1969) supplemented with either 0.2% glucose or 0.4% lactose, 50 μg/ml of the appropriate required amino acids, and, for plates, 2% agar. Guanosine was added to LB and minimal media at 25 μg/ml. The antibiotics ampicillin (Ap), streptomycin (Sm), tetracycline (Tc), chloramphenicol (Cm), and kanamycin (Km) were added at concentrations of 100, 100, 15, 15, and 60 μg/ml, respectively. LCG media for preparation of P1 lysates and transductions consisted of LB media supplemented with 2 mm calcium chloride, 50 μg/ml thymine, 0.2% glucose, and 1.2% agar; LCG top agar contained 0.7% agar. P1 transductions were performed as described (Miller 1992). Media for mutation assays contained either rifampicin (Rif) or nalidixic acid (Nal) at 100 or 20 μg/ml, respectively. Serial dilutions employed 56/2 buffer.

Mutation assays: Mutation to Rif-resistance, Nal-resistance, and leucine (Leu)-prototrophy was determined for eight independent cultures in 2-ml LB media after overnight growth. For assays scoring Rif-resistance, independent cultures were plated directly onto LB + Rif. For selection of Nal-resistance and Leu$^+$, cells from overnight cultures were washed twice in 56/2 buffer and subsequently plated onto LB + Nal or minimal media lacking leucine. Total viable cells were determined by serial dilution with 56/2 buffer followed by plating on either LB, for the Rif and Nal assays, or on minimal-complete media for Leu assays. For the Rif and Nal assays, colonies were counted after overnight growth at 37°. For Leu assays, plates were scored after 3 days growth. Mutation rates were calculated by the method of the median (Lea and Coulson 1949) using the following formula: mutation rate $= M/N$, 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 r_0, the median number of mutant cells determined among the cultures, by the formula $r_0 = M(1.24 + \ln M)$. For the Leu assay, mutation rates were calculated by the maximal likelihood method based on the number of cultures showing zero revertants (Lea and Coulson 1949).

The frequency of specific spontaneous base substitutions and frameshift mutations was determined using a *lacZ* detection system (Cupples and Miller 1989; Cupples *et al.* 1990). Briefly, F′ episomes were conjugally transferred from the 11 CC strains (CC101–CC111) into Δ*lac* derivatives of wild type (STL3678) or the triple exonuclease-deficient mutant (STL3616), selecting Pro$^+$ Sm^r. For reversion assays, individual colonies freshly grown on minimal medium lacking proline were inoculated into 2 ml of LB liquid media and incubated at 37° for 18–24 hr. The numbers of Lac$^+$ revertants were determined by plating onto lactose-minimal medium and compared to the numbers of F′ cells in the culture determined by plating to minimal medium lacking proline. Colonies were counted after 48 hr of growth; average reversion frequencies are reported.

Plasmid frameshift assays: Plasmids pXVIII and pXIX (Hoffman *et al.* 1996) are *tetA*$^-$ pBR322-derivative plasmids with single-bp alterations within the *tetA* gene to allow for the detection of ±1 frameshift mutations. Both plasmids contain alterations within a guanine nucleotide repeated sequence (GGGGG) found at position 536-540 of pBR322. pXIX contains a +1 frameshift of this sequence, whereas pXVIII contains a −1 frameshift; a compensatory frameshift restores the correct reading frame and therefore Tc-resistance to the cells. In wild-type strains, almost all compensatory mutations occur within the G run itself (Maenhaut-Michel *et al.* 1992). Plasmids were introduced into various strains by the Mg^{2+}-polyethylene glycol-dimethyl sulfoxide transformation method (Chung *et al.* 1989) by selection for Ap-resistance. Because of the low spontaneous frameshift rates exhibited in these assays, frameshift mutations were stimulated by growth in the presence of the frameshift mutagen ICR-191 (Sigma, St. Louis) at 20 μg/ml. For each set of assays, eight independent transformants were innoculated into 2-ml LB + Ap + ICR-191 liquid media and incubated at 37° for 18 hr in the dark. To determine the number of Ap^r and Tc^r colonies, serial dilutions were spotted on LB + Ap and LB + Ap + Tc plates and incubated overnight at 37°. Frameshift rates were calculated by the method of the median (Lea and Coulson 1949).

UV survival assays: Cells were grown in LB liquid media to exponential stage (OD_{600} = 0.4–0.5), serially diluted in 56/2 buffer, and plated on LB agar plates. Plates were immediately irradiated with 20, 40, and 80 J/m^2 doses of UV (254 nm) irradiation and incubated at 37° in the dark overnight. Total viable cells were determined from serially diluted unirradiated cells.

Conjugational recombination: All mutants were assayed for conjugal inheritance with the Exo$^+$ control strain in parallel. Matings were performed for 30 min at 37° with a 10:1 recipient-to-donor ratio using recipient cells grown to an OD_{600} of 0.4 and donor cells grown to an OD_{600} of 0.3. After mating, the cells were mixed vigorously for 1 min, serially diluted in 56/2 buffer, and plated on appropriate media for selection of recombinants. When possible, the Hfr JC11033 was used to assay inheritance of both a chromosomal marker, *leuB*, by recombination and a ColE1 plasmid marker, *kan*, conjugally transferred in the same cross. Contraselection in all cases was Ser$^+$ Sm^r in minimal media.

RESULTS

The role of ssExos in mutation avoidance: Null mutations in the genes for RecJ exonuclease (*recJ*), Exonuclease I (*xonA*), and Exonuclease VII (*xseA*) were introduced singly and in combination into a standard genetic background (Table 1). Spontaneous mutation rates were measured by several assays: mutation to Rif-resistance, Nal-resistance, and reversion of the *leuB6* allele. No significant increase in mutation rates was observed in any combination of ssExo mutations (Table 2). This contrasts to large increases observed for mutants in known components of the methyl-directed mismatch repair pathway, including *mutS* and *uvrD*. MutS protein is essential for mismatch recognition, and the UvrD helicase is believed to unwind DNA from the incision site to the mismatch to allow excision of the mismatched strand (Modrich 1991).

We also assayed the triple RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutant using the set of F *lac* elements (Cupples and Miller 1989) that score specifically for each of the six base substitution mutations. The triple ssExo$^-$ mutant had no detectable effect on any of the six base substitutions as compared to the wild-type control strains (Table 3). Reported transition mutation frequencies (in strains

TABLE 1

***Escherichia coli* K-12 strains**

Strain	Genotype	Source or derivation
	A. Strains related to BT199[a]	
BT199	F$^-$ λ$^-$ *thi-1* Δ(*gpt-proA*)*62 thr-1 leuB6 kdgK51 rfbD1 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31 rac*$^-$.	W. Wackernagel
HR839	+ Δ*xonA300::cat argE3 hisG4*	(Razavy *et al.* 1996)
JC12123	+ *recJ284::Tn10 argE3 hisG4*	(Lovett and Clark 1984)
RDK1445	+ *zgb-224::Tn10 serA6 argE3 hisG4*	R. Kolodner
STL113	+ *recJ2052::Tn10kan argE3 hisG4*	Ser$^+$ Km^r Tc^s transductant P1 STL125 × RDK1445
STL125	+ *recJ2052::Tn10kan recD1013 argE3 hisG4*	This study[a]
STL1526	+ *uvrD254::Tn5 argE3 hisG4*	(Lovett and Sutera 1995)
STL2331	+ *recJ284::Tn10*	Tc^r transductant P1 JC12123 × BT199
STL2347	+ Δ(*xseA-guaB*) *zff-3139::Tn10kan*	Km^r transductant P1 WF3121 × BT199
STL2348	+ Δ(*xseA-guaB*) *zff-3139::Tn10kan recJ284::Tn10*	Km^r transductant P1 STL2347 × STL2331
STL2694	+ Δ*xonA300::cat*	Cm^r transductant P1 HR839 × BT199
STL2700	+ Δ(*xseA-guaB*) *zff-3139::Tn10kan recJ284::Tn10* Δ*xonA300::cat*	Cm^r transductant P1 STL2694 × STL2348
STL2729	+ Δ*xonA300::cat recJ284::Tn10*	Cm^r transductant P1 STL2694 × STL2331
STL2758	+ Δ*xonA300::cat* Δ(*xseA-guaB*) *zff-3139::Tn10kan*	Cm^r transductant P1 STL2694 × STL2347
STL2935	+ Δ*xonA300::cat* Δ(*xseA-guaB*) *zff-3139::Tn10kan serA6 zgb-224::Tn10*	Tc^r Ser$^-$ transductant P1 RDK1445 × STL2758
STL2971	+ Δ*xonA300::cat* Δ(*xseA-guaB*) *zff-3139::Tn10kan recJ2052::Tn10kan*	Ser$^+$ transductant P1 STL113 × STL2935
STL773	+ *mutS201::Tn5 argE3 hisG4*	R. Kolodner
STL3185	+ *cysC95::Tn10* Δ*xonA300::cat* Δ(*xseA-guaB*) *zff-3139::Tn10kan*	Tc^r transductant P1 CAG12173 × STL2758
STL3187	+ *mutS201::Tn5* Δ*xonA300::cat* Δ(*xseA-guaB*) *zff-3139::Tn10kan*	Cys$^+$ Mut transductant P1 STL773 × STL3185
STL3240	+ *mutS201::Tn5*	Kn^r transductant P1 STL773 × BT199
STL3629	+ *gpt*$^+$ *pro*$^+$	Pro$^+$ transductant P1 CAG18427 × BT199
STL3675	+ *proC::Tn5kan zah-281::Tn10*	Tc^r Pro$^-$ transductant P1 ER2562 × STL3629
STL3677	+ Δ(*lacI-200lacA*)	Pro$^+$Tc^{s}Lac$^-$ transductant P1 ER2580 × STL3613
STL3678	+ *proAB81::Tn10*	Tc^r transductant P1 CAG18447 × STL3677
STL3679-STL3689	+ F′ *lacI*$^-$*Z*$^-$ *proAB*$^+$	STL3678 Pro$^+$ Sm^r recipient of F′ from CC101-111[b]
STL3478	+ *gpt*$^+$*pro*$^+$ Δ*xonA300::cat* Δ(*xseA-guaB*) *zff-3139::Tn10kan recJ2052::Tn10kan rac*$^-$.	Pro$^+$ transductant P1 CAG18427 × STL2971
STL3613	+ *proC::Tn5kan zah-281::Tn10*	Tc^r Pro$^-$ transductant P1 ER2562 × STL3478
STL3614	+ Δ(*lacI-200lacA*)	Pro$^+$Tc^{s}Lac$^-$ transductant P1 ER2580 × STL3613
STL3616	+ *proAB81::Tn10*	Tc^r transductant P1 CAG18447 × STL3614
STL3618-STL2628	+ F′ *lacI*$^-$*Z*$^-$ *proAB*$^+$	STL3678 Pro$^+$ Sm^r recipient of F′ from CC101-111[b]

(*continued*)

TABLE 1

Continued

Strain	Genotype	Source or derivation
	B. Other	
CAG12173	*cysC95::Tn10*	C. Gross
CAG18447	*proAB81::Tn10*	C. Gross
CAG18427	*zje-2241::Tn10*	C. Gross
CC101-CC111	F′ *lacI*$^-$*Z*$^-$ *proAB*$^+$ *ara* Δ(*lacproB*)$_{X111}$	(Cupples *et al.* 1990)
ER2562	F$^-$λ$^-$ *proC::Tn5 zah-281::Tn10* IN(*rrnD-rrnE*) *rph-1*	E. Raleigh
ER2580	F$^+$λ$^-$ Δ(*lacI-200lacA*)	E. Raleigh
JC158	Hfr PO1 *thr-1 serA6 relA1 lacI22*	(Clark 1963)
JC11033	Hfr PO1 pML2 *thi-1 serA6 relA1 lacI22*	(Lovett 1983)
WF3121	Δ(*xseA-guaB*) *zff-3139::Tn10kan uvrA6* Δ(*lac-pro*)*XIII galK2 rpsL* λ*cI ind-*	J. Hayes

[a] STL125 is a recombinant of RDK1792 (*recD1013*, Lovett *et al.* 1988) with transformed plasmid pRDK162 cleaved with *Pst*I, *Sal*I, and *Eco*RI. pRDK162 is a Tn*10kan* insertion mutant of plasmid pJC763 (Lovett and Clark 1985) isolated as described (Way *et al.* 1988) with λNK1105 provided by N. Kleckner. The transposon insertion point in *recJ2052::Tn10kan* is coincident with the insertion point of *recJ284::Tn10* 481 bp downstream from the GTG start of the *recJ* coding sequence.

[b] Strains STL3618-STL3628 and STL3679-STL3689 are derivatives of the strains STL3616 and STL3678, respectively, carrying an F′ *lacI*$^-$*Z*$^-$ *proAB*$^+$ episome introduced by mating with donor strains CC101–CC111, selecting Pro$^+$ Sm^r. Each strain carries a different mutation in *LacZ*, described in Table 3 (see also Cupples *et al.* 1990).

CC102 and CC106) for mismatch repair-deficient *mutH* derivatives are about 100-fold higher than wild-type strains (Cupples and Miller 1989).

However, in *lac* reversion assays scoring +1 and −1 frameshift mutations in nucleotide "runs" (Cupples *et al.* 1990), the RecJ$^-$ ExoI$^-$ ExoVII$^-$ strain showed significant elevations, from 5- to 18-fold greater than that observed with wild-type strains (Table 3). Reversion of a GC dinucleotide frameshift was not strongly affected. Reported frameshift frequencies for mismatch repair-defective MutH$^-$ strains are somewhat higher than those observed for the ssExo mutant: 30–40-fold higher for the +1 frameshifts and five- to ninefold higher for the −1 frameshifts (Cupples *et al.* 1990). (The −2 dinucleotide frameshift was not tested.)

TABLE 2

Mutation rates in exonuclease mutants

Strain	Description	Mutation rate[a] × 10^9 Rif^r	Nal^r	Leu$^+$
BT199	Exo$^+$	10	1.5	0.38
STL2694	ExoI$^-$	10	1.5	0.52
STL2347	ExoVII$^-$	8.4	4.3	0.09
STL2331	RecJ$^-$	4.6	1.6	0.56
STL2758	ExoI$^-$ ExoVII$^-$	3.4	0.4	0.17
STL2729	ExoI$^-$ RecJ$^-$	4.4	1.0	0.75
STL2348	ExoVII$^-$ RecJ$^-$	6.0	0.3	0.07
STL2700	ExoI$^-$ ExoVII$^-$ RecJ$^-$	17	0.2	0.34
STL1526	UvrD$^-$	120	32	2.1
STL773	MutS$^-$	220	330	5.1

[a] Mutation rates were determined as described in materials and methods. Reported rates for each strain represent averages among at least two sets of eight independent cultures for Rif and Nal and one set for Leu.

To examine frameshift mutagenesis in more detail, we employed a plasmid-based assay specific for frameshift mutations (Hoffman *et al.* 1996). Plasmids that carried a *tetA* frameshift mutation in a run of five G residues were introduced into various strains carrying combinations of ssExo mutations. Because spontaneous rates were extremely low, reversion to Tc-resistance by compensatory +1 or −1 frameshifts was scored after addition of the acridine ICR-191. ICR-191 specifically stimulates frameshift mutations, presumably by stabilizing the extrahelical base in a misaligned intermediate (Ferguson and Denny 1990). ExoVII mutants show a modest increase in both +1 and −1 frameshift mutation rates, and the double ExoI$^-$ ExoVII$^-$ mutant was dramatically enhanced (Table 4). The synergy between ExoI and ExoVII may mean that these exonucleases play a redundant role in the avoidance of frameshift mutations. Loss of the RecJ exonuclease had no effect on the +1 assay and a very slight effect on the −1 assay. Mutations in MutS also elevated frameshift mutagenesis, although in the +1 assay its effect was not as great as that of the ExoI$^-$ ExoVII$^-$ strain (Table 4). The triple MutS$^-$ ExoI$^-$ ExoVII$^-$ mutant exhibited frameshift mutagenesis rates that approximated that of the MutS$^-$ single mutant. For the +1 frameshift assay, this means that a MutS$^-$ mutation actually suppresses much of the mutability of the ExoI$^-$ ExoVII$^-$ strain.

The role of ssExos on UV repair and homologous recombination: Mutants in RecJ, ExoI, and ExoVII were tested for survival after UV irradiation (Figure 1). Single and double mutants in ExoI and ExoVII showed UV survival comparable to wild-type strains. Single RecJ$^-$

TABLE 3

Spontaneous Lac$^+$ revertants per 10^8 cells

Base substitutions					Frameshifts				
Episome from:	Reversion event	ssExo$^+$	ssExo$^-$	Rel. freq/ (Exo$^-$/Exo$^+$)	Episome from:	Reversion event	ssExo$^+$	ssExo$^-$	Rel. freq. (Exo$^-$/Exo$^+$)
CC101	A·T→C·G	1.3	0.51	0.39	CC107	+1G	42	300	7.2
CC102	G·C→A·T	1.2	1.7	1.42	CC108	−1G	50	910	18
CC103	G·C→C·G	0.50	0.40	0.82	CC109	−2GC	110	160	1.5
CC104	G·C→T·A	1.8	1.4	0.77	CC110	+1A	1.4	7.2	5.2
CC105	A·T→T·A	0.76	0.40	0.48	CC111	−1A	6.0	55	9.3
CC106	A·T→G·C	0.64	0.38	0.60					

STL3679-STL3689 (ssExo$^+$) and STL3618-STL3628 (ssExo$^-$; ExoI$^-$ ExoVII$^-$ RecJ$^-$) carry F′ episomes derived from the indicated strain, each with a different mutant version of *lacZ*. These Lac$^-$ strains can revert to Lac$^+$ by a mutation of the specified type, as described (materials and methods; Cupples *et al.* 1990). Reported frequencies for each strain are the average of at least five independent assays, as described in materials and methods.

and double RecJ$^-$ ExoI$^-$ mutants exhibited slight sensitivity to UV. Addition of a mutation in ExoVII enhanced UV-killing in both RecJ$^-$ and RecJ$^-$ ExoI$^-$ mutants. The triple RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutant was extremely sensitive to UV. RecJ exonuclease appears to be required for UV repair but can be replaced by ExoVII (with a common 5′ polarity) and somewhat less so by ExoI (with a 3′ polarity).

Homologous recombination was measured by the inheritance of the *leuB*$^+$ marker after conjugation (Table 5). RecJ$^-$ and ExoVII$^-$ strains were not significantly affected in their ability to inherit *leuB* by conjugation. ExoI$^-$ mutants were two- to threefold depressed for inheritance of *leuB*. Double mutants in ExoI and RecJ recombined 10–12-fold less efficiently than wild-type strains. Inheritance of another marker, *kan*, conjugally transferred on ColE1, which does not require homologous recombination, was normal in these strains, suggesting a true reduction of recombination, rather than an inability to conjugate. The addition of an ExoVII mutation may have increased the recovery of recombinants somewhat (twofold) in both ExoI$^-$ and ExoI$^-$ RecJ$^-$ strains. These results suggest that either ExoI or RecJ are required for optimal levels of homologous recombination; ExoVII cannot substitute and in fact may interfere with this role.

DISCUSSION

Genetic analysis of ssExo mutants in *E. coli* reveals complex and partially redundant roles for these exonucleases in several aspects of DNA metabolism. Multiple mutants for the three ssExos, RecJ exonuclease, Exonuclease I, and Exonuclease VII, appeared largely proficient in methyl-directed mismatch correction. Spontaneous base substitution mutations measured at several loci were not increased in the triple mutant, in contrast to large increases seen for mutants in MutS and UvrD (Helicase II). If these exonucleases are required for mismatch excision *in vivo*, then the action of an additional exonuclease(s) must suffice to substi-

TABLE 4

Enhanced frameshift mutation rate of exonuclease mutants

		Rate × 10^7	
Strain	Description	+1 Frameshifts	−1 Frameshifts
BT199	Exo$^+$	8.5	3.7
STL2694	ExoI$^-$	10	5.2
STL2347	ExoVII$^-$	55	27
STL2758	ExoI$^-$ ExoVII$^-$	270	42
STL2971	ExoI$^-$ ExoVII$^-$ RecJ$^-$	300	87
STL3240	MutS$^-$	50	31
STL3187	MutS$^-$ ExoI$^-$ ExoVII$^-$	32	42

Frameshift assay plasmids pXVII and PXIX (Hoffman *et al.* 1996) were transformed into the strains indicated above. +1 and −1 frameshift formation was assayed by plating to Tc-medium. Twenty μg/ml ICR-191 was added to cultures during preplating growth. Mutation rates were determined by the method of the median as described in materials and methods. Reported rates for each strain are averages of two sets of eight independent cultures.

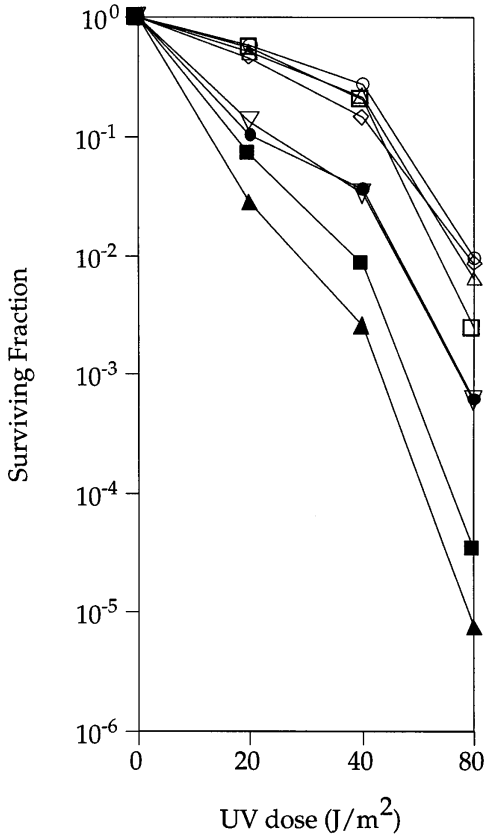

Figure 1.—Synergistic effect of single-strand exonuclease mutants on UV survival. Shown are UV survival curves of various combinations of single-strand exonuclease mutants in the BT199 background. BT199, Exo$^+$ (□); STL2694, ExoI$^-$ (◇); STL2347, ExoVII$^-$ (○); STL2331, RecJ$^-$ (▽); STL2758, ExoI$^-$ ExoVII$^-$ (△); STL2729, ExoI$^-$ RecJ$^-$ (●); STL2348, ExoVII$^-$ RecJ$^-$ (■); STL2700, ExoI$^-$ ExoVII$^-$ RecJ$^-$ (▲).

tute for the loss of the others. Biochemical experiments also point to the existence of an additional, unspecified, 3′ exonuclease functional for mismatch repair (Cooper *et al.* 1993). One might imagine that proofreading 3′ exonucleases associated with the three DNA polymerases in *E. coli* might carry out this role. However, neither purified Polymerase I nor Polymerase III could provide 3′ excision activity *in vitro* (Cooper *et al.* 1993); Polymerase II was not tested.

Despite our failure to detect effects on base substitutions, reversion of several frameshift mutations in *lacZ* was stimulated in the RecJ$^-$ ExoI$^-$ ExoVII$^-$ triple mutant relative to wild-type strains. The magnitude of this effect varied, but stimulation was observed at loci with both low and high basal reversion rates. A more detailed genetic analysis showed that acridine-induced +1 and −1 frameshift mutations occurred 20–30-fold more frequently in ExoI$^-$ ExoVII$^-$ strains. A single ExoVII mutation had a modest effect on frameshift mutagenesis that was exacerbated by an additional mutation in ExoI. This synergy suggests that ExoI and ExoVII may function redundantly in the avoidance of frameshift mutations. RecJ had little additional effect on this phenomenon.

It is possible that this elevation of frameshift mutagenesis is because of loss of methyl-directed mismatch repair capacity, and, for some reason, frameshift mutagenesis is more sensitive than base substitution mutagenesis. Because recognition of both base substitution and frameshift mismatches is mediated by the same protein, MutS (Modrich 1991), it seems unlikely that the excision machinery is differentially specified by the mismatch type. In general, the frameshift mutations we assayed in *lacZ* (Table 3) occurred at higher frequencies than base substitution mutations in the same locus and may be more sensitive indicators of mismatch repair deficiency. However, one frameshift (with the CC110 F′ element) occurred at lower frequencies (similar to that seen for the base transition mutations) and was nonetheless stimulated fivefold in the ssExo mutant. Moreover, loss of MutS actually reduced the recovery of +1 frameshift mutations in the ExoI$^-$ ExoVII$^-$ strain, suggesting that the methyl-directed mismatch repair pathway in some way promotes frameshift mutagenesis in ExoI$^-$ ExoVII$^-$ mutants. If a frameshift mutation were to arise on a methylated template strand or on fully unmethylated DNA produced during recombination or repair, methyl-directed mismatch repair could remove its wild-type counterpart on an unmethylated DNA strand and therefore aid in the establishment of the frameshift. Alternatively, some frameshift mutations sensitive to ExoI and ExoVII may occur during the DNA synthesis that accompanies mismatch repair itself. Nonreplicative frameshift mutagenesis from processing of topoisomerase-induced nicks has been reported by Ripley and coworkers for bacteriophage T4 (Masurekar *et al.* 1991; Kaiser and Ripley 1995). Displaced ssDNA, susceptible to exonucleolytic degradation by ExoI or ExoVII, may be an intermediate in some slipped realignment reactions that give rise to frameshift mutations in repetitive DNA runs (Figure 2). Susceptibility to ssExos may distinguish nonreplicative or repair-replication slipped realignments from those that occur during normal DNA replication where the presence of bound DNA polymerase would preclude their access.

The most profound effect of the ssExos was on survival of UV irradiation where ssExos provided a partially redundant function. RecJ$^-$ mutants showed a slight sensitivity to UV at high doses; ExoI$^-$ and ExoVII$^-$ single and double mutants had no detectable effect. However, the combination of RecJ and ExoVII mutations resulted in extreme sensitivity to UV. The triple RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutant was the most sensitive derivative. These results indicate both specialization and redundancy of these ssExos for UV repair. The nucleases with 5′ polarity, RecJ and ExoVII, seem most important for repair, with RecJ playing a slightly more important role. In the absence of RecJ and ExoVII, ExoI can promote some measure of protection from UV, as indicated by the fact that triple RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutants are somewhat more sensitive than RecJ$^-$ ExoVII$^-$ double mutants.

What function are the ssExos providing for UV repair?

TABLE 5

Conjugational inheritance in exonuclease mutants

Strain	Designation	Relative recombination frequency ×JC158	*n*	Relative recombination frequency ×JC11033	*n*	Relative plasmid transfer ×JC11033	*n*
BT199	Exo$^+$	(1)	13	(1)	6	(1)	4
STL2694	ExoI$^-$	0.42 ± 0.20	9	0.28 ± 0.18	6	1.2 ± 0.23	4
STL2347	ExoVII$^-$	0.91 ± 0.23	9	ND		ND	
STL2331	RecJ$^-$	0.88 ± 0.36	13	0.88 ± 0.34	6	0.91 ± 0.11	4
STL2758	ExoI$^-$ ExoVII$^-$	0.90 ± 0.35	7	ND		ND	
STL2729	ExoI$^-$ RecJ$^-$	0.10 ± 0.07	11	0.07 ± 0.02	6	0.92 ± 0.14	4
STL2348	ExoVII$^-$ RecJ$^-$	0.60 ± 0.23	9	ND		ND	
STL2700	ExoI$^-$ ExoVII$^-$ RecJ$^-$	0.28 ± 0.10	7	0.20 ± 0.12	4	ND	

Matings were performed as described in materials and methods with designated recipient above and either JC158 or JC11033 as donor. Recombinants were selected by Thr$^+$ Leu$^+$ [Ser$^+$ Sm^r] and plasmid transfer by Km^r[Ser$^+$ Sm^r]. Inheritance frequencies are expressed relative to that determined for BT199, which was 1–8% for chromosomal markers and 1–6% for plasmid transfer. Standard deviations of the mean are given for both relative recombination frequencies and for relative plasmid transfer. ND, not determined; *n*, number of determinations of relative frequency.

These strains are sensitive to UV doses at which many pyrimidine dimers are introduced into the *E. coli* chromosome (with 1000–1200 dimers/genome estimated at 20 J/m^2) (Witkin 1976; Gruskin and Lloyd 1988). The inability to repair any one of them would presumably result in death. General excision of pyrimidine dimers is provided by the UvrABC complex (Van Houten 1990), and *uvrABC* mutants are profoundly sensitive to UV. Mutations in *uvrA* and *recJ* are synergistic for their effects on UV survival (Lovett 1983). We suggest that the ssExos are required for repair of a specific class of dimers, perhaps those that arise in the replication fork (Figure 3). Such dimers will block DNA polymerization and cannot be excised by UvrABC excinuclease because of their single-stranded nature. Single-strand gaps in the daughter strands are observed after UV irradiation and are subsequently filled by recombination in a process termed "postreplication DNA repair" (Rupp *et al.* 1971; Rupp 1996). We propose that at least some postreplication repair reactions require ssExo action, perhaps to widen the gaps so that RecA filament formation can be initiated (Figure 3) or to stabilize the recombinational strand exchange reactions that follow (Figure 4). This could potentially be more important for repair of lesions on the lagging strand where gaps between the lesion and the previous Okazaki fragment may be, in some cases, quite short (Figure 3).

A requirement for ssExos in UV-induced recombinational events *in vivo* has been previously described. UV-induced recombination between nonreplicating λ phage is reduced by mutations in RecJ, ExoI, or ExoVII (Feng and Hays 1995). Some redundancy of ssExo

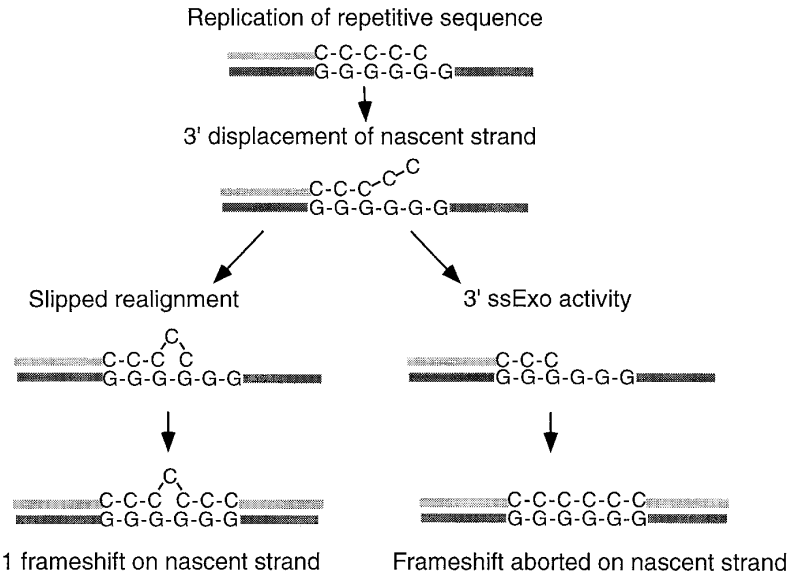

Figure 2.—Degradation of frameshift strand slippage intermediates by 3′ ssExos. Frameshift mutations are often clustered in monotonic nucleotide repeat sequences and have been proposed to result from slipped realignment during DNA replication (Streisinger *et al.* 1966). Dissociation of the one strand from its complement precedes its realignment into the slipped configuration. (Frameshift mutagens such as ICR-191 stabilize the unpaired base in the slipped structure, Ferguson and Denny 1990.) If not repaired, the slippage shown on the left will produce a +1 frameshift mutation. However, the displaced 3′ strand is subject to degradation by ssExos such as Exonuclease I and Exonuclease VII. Such degradation should abort slipped realignment and subsequent frameshift mutations.

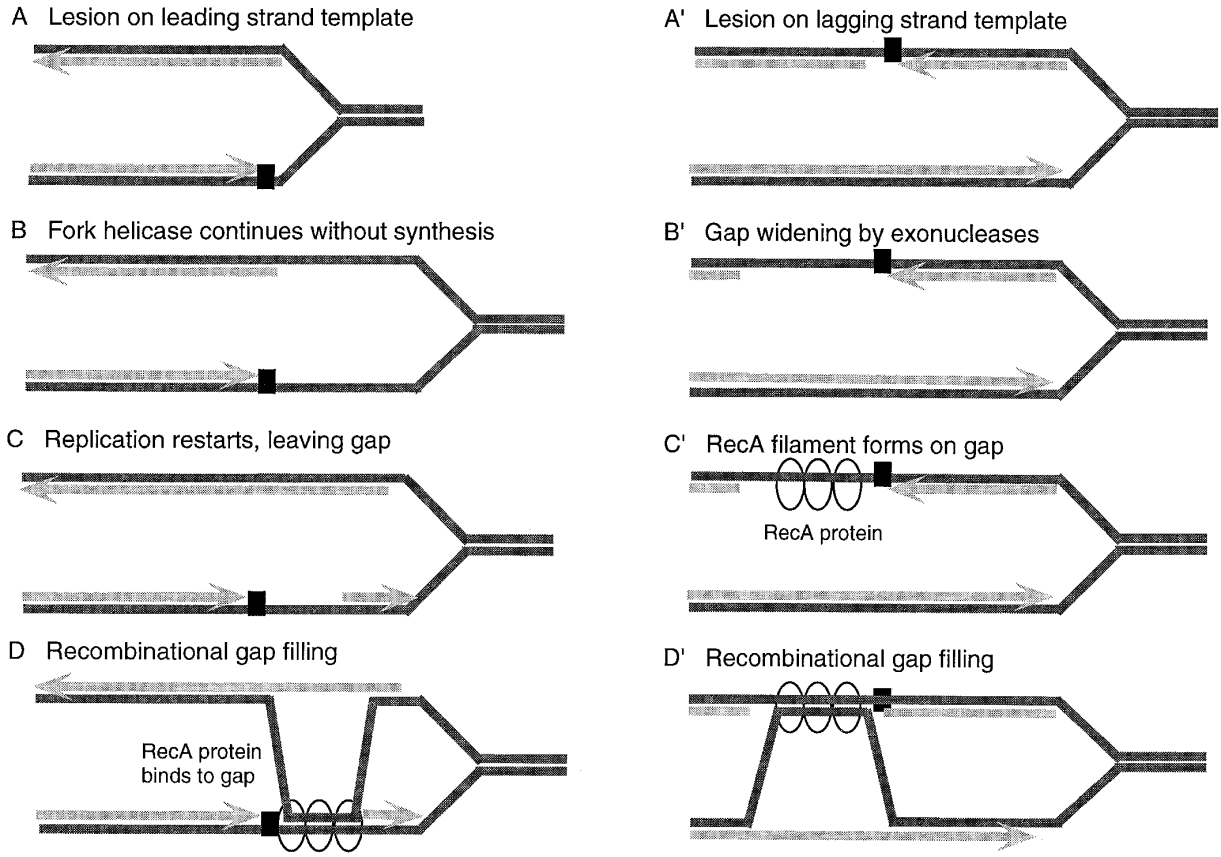

Figure 3.—Postreplication repair of replication-blocking lesions (Rupp 1996). (A) A lesion such as a UV-induced pyrimidine dimer blocks synthesis of the leading strand. (B) Movement of the replication fork helicase becomes uncoupled from DNA synthesis. (C) Replication restarts but a gap remains. (D) Gap is filled by RecA-dependent recombination. The ssExos may aid in this reaction, as shown in Figure 4. (A′) A lesion blocking lagging strand synthesis may result in a very short gap if the lesion is close to the 5′ end of the previous Okazaki fragment. (B′) Before recombinational repair can proceed, it may be necessary to widen the gap by exonuclease action. The ssExos, coupled with DNA unwinding, may accomplish this step. (C′) Once the gap is sufficiently large, RecA protein can form a filament to initiate synapsis. (D′) Recombinational repair fills the gap, perhaps aided additionally by ssExos, as in Figure 4.

function was evident, as in our analysis, because any double mutants resulted in almost a complete loss of this type of recombination. However, in these experiments loss of any single exonuclease produced a reduction, unlike what we observed for UV survival. In addition, mismatch repair genes are required for UV-induced recombination between nonreplicating λ (Feng and Hays 1995) but not for UV survival in *E. coli* (S. T. Lovett, unpublished results).

Although the triple ssExo mutant produced a strong deficiency in UV survival that approximated that of mutants for the RecBCD nuclease/helicase complex (Emerson and Howard-Flanders 1967), no dramatic loss of recombination capacity was evident. Mutations in the RecBCD complex reduce recombination measured after conjugation ~100-fold in a similar assay (Lovett *et al.* 1988); triple RecJ$^-$ ExoI$^-$ ExoVII$^-$ mutants were reduced as shown here about three- to five-fold. We did, however, observe a consistently modest reduction in recombination levels (10–14-fold) in double mutants for RecJ and ExoI, indicating that these exonucleases may enhance somewhat the recovery of recombinants via the RecBCD pathway. A similar requirement for either ExoI or RecJ has also been noted in recombination between λ bacteriophage mediated by the RecBCD pathway (Razavy *et al.* 1996). After P22 transduction in *Salmonella typhimurium*, ExoI$^-$ RecJ$^-$ mutants also exhibit a slight reduction in recombination (Miesel and Roth 1996). When the region of homology between the transducing DNA and the chromosome is restricted, a much more dramatic reduction of recombination is seen, suggesting that the function of either Exonuclease I or RecJ exonuclease is required for optimal RecBCD-dependent recombination under these conditions (Miesel and Roth 1996).

The ssExos may play a role in stabilizing the joint molecules formed during strand exchange by digestion of certain DNA strands (Figure 4). *In vitro* strand exchange reactions also support a stimulatory role of ssExos in recombination. RecJ exonuclease enhances RecA-mediated strand exchange in reactions between single-strand circular and duplex linear homologous

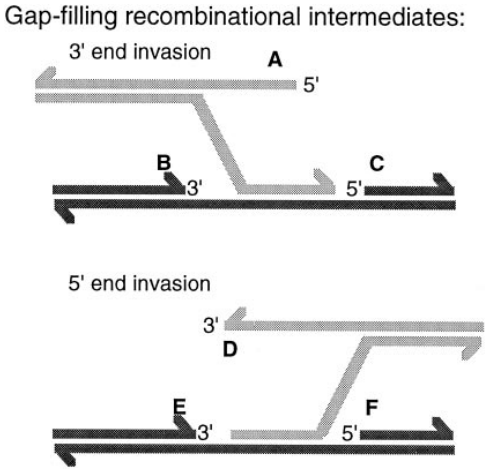

Figure 4.—Recombination joints and their stabilization by ssExos. Widening of the gaps (B–C and E–F) on the recipient chromosomes by exonuclease action may facilitate RecA binding and initiation of synapsis and strand exchange. 3′ end invasion, or alternatively 5′ end invasion, into a gap may produce heteroduplex intermediates similar to those illustrated here. Postsynaptically, such intermediate structures may be further stabilized by exonucleolytic degradation. If the donor chromosomes have been broken (as shown), degradation of 5′ end A or 3′ end D will remove a competitor strand for pairing. Digestion of 3′ end B or 5′ end F will allow further strand uptake and extend the heteroduplex region. Degradation of 5′ strand C will stabilize the 3′ invasion heteroduplex by allowing DNA polymerization to extend the region of pairing in the joint.

DNA substrates (Corrette-Bennett and Lovett 1995). By degrading the 5′ strand that competes for pairing, RecJ accelerates the 3′ to 5′ uptake of one strand of the duplex substrate molecule (Corrette-Bennett and Lovett 1995). ExoI has been shown *in vitro* to allow RecA to promote transfer of 5′ ends, again presumably by removing the 3′ competitor strand for pairing in these reactions (Konforti and Davis 1992; Bedale *et al.* 1993).

Exonuclease VII was not required for recombinant formation after conjugation in our experiments; rather, ExoVII$^-$ derivatives of RecJ$^-$ ExoI$^-$ consistently gave slightly higher levels of recombination than RecJ$^-$ ExoI$^-$ derivatives. A hyper-recombinational phenotype for ExoVII$^-$ mutants has been previously observed (Chase and Richardson 1977). Unlike the case for UV survival where ExoVII appeared to be able to substitute for RecJ, ExoVII apparently cannot provide the function of RecJ during recombination reactions. The reason for this discrepancy is not clear. One potential explanation may be an inhibitory effect of single-strand binding protein (SSB) on ExoVII degradation during recombination reactions *in vivo*. Binding of SSB to ssDNA does not inhibit either ExoI or RecJ exonucleases *in vitro*; in fact, SSB stimulates degradation by the exonucleases (Molineux and Gefter 1975; S. T. Lovett, unpublished results). The effect of SSB on ExoVII degradation has not been reported. Alternatively, specific protein interactions may favor RecJ or ExoI participation in recombinational reactions or preclude action of ExoVII.

The redundant role of ssExos in several DNA metabolic processes reinforces the value of examining multiple mutants for phenotypic effects. It has always been somewhat puzzling why RecJ exonuclease mutants are not especially sensitive to UV irradiation, unlike mutants in RecF, RecR, and RecO proteins that together with RecJ define the "RecF pathway" for recombination (Clark 1991). The reason for this disparity is that RecJ can be replaced, at least for UV repair, by Exonuclease VII. Likewise, the strong frameshift mutator phenotype exhibited by double mutants in Exonuclease I and VII is not apparent in either single mutant.

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