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Two Enzymes, Both of Which Process Recombination Intermediates, Have Opposite Effects on Adaptive Mutation in *Escherichia coli*

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ABSTRACT

Reversion of a *lac*⁻ frameshift allele carried on an F′ episome in *Escherichia coli* occurs at a high rate when the cells are placed under lactose selection. Unlike Lac^+ mutations that arise during nonselective growth, the production of these adaptive mutations requires the RecA-RecBCD pathway for recombination. In this report, we show that enzymes that process recombination intermediates are involved in the mutagenic process. RuvAB and RecG, *E. coli*'s two enzymes for translocating Holliday junctions, have opposite effects: RuvAB is required for RecA-dependent adaptive mutations, whereas RecG inhibits them.

MUTATIONS can arise in static populations of bacterial cells. In some cases, the mutational process appears to be adaptive in that the only mutations recovered are those that allow the cells to grow (CAIRNS *et al.* 1988; FOSTER 1993). There may be many mechanisms by which mutations can be generated in nondividing cells, but studies of adaptive mutation in a particular strain of *Escherichia coli*, FC40, have revealed a novel mutagenic pathway. FC40 is deleted for the *lac* operon on its chromosome and carries a F′ episome with a mutant *lac* allele. The allele, Φ(*lacI33-lacZ*), derives from a fusion of *lacI* to *lacZ* and has a +1 base-pair frameshift in the *lacI* sequence (CALOS and MILLER 1981; MILLER 1985). When FC40 is plated on medium with lactose as the sole energy and carbon source, reversion to Lac^+ occurs at a high rate with new Lac^+ colonies (postplating mutations) appearing daily (CAIRNS and FOSTER 1991). Unlike the Lac^+ mutations that occur during nonselective growth, the postplating Lac^+ mutations depend on the RecA-RecBCD pathway for recombination (CAIRNS and FOSTER 1991; HARRIS *et al.* 1994). In addition, although a variety of mutations can restore the Lac^+ phenotype during growth, the postplating mutations are dominated by simple one-base deletions, the majority of which are at runs of iterated bases (FOSTER and TRIMARCHI 1994; ROSENBERG *et al.* 1994). This latter result suggests that the postplating mutations arise as errors made during DNA synthesis, and we have recently shown that, in wild-type cells, DNA polymerase III is responsible for most of these mutations (FOSTER *et al.* 1995). Because RecA-dependent adaptive reversion of *lacI33-lacZ* requires that the allele be on the episome and that certain conjugal functions be expressed, it has been proposed that the DNA synthesis initiated at *oriT*, the F conjugal origin, generates the mutations (FOSTER and TRIMARCHI 1995; GALITSKI and ROTH 1995; RADICELLA *et al.* 1995). However, it is just as possible that conjugal functions are required to produce substrates for recombination, and that DNA synthesis associated with this recombination generates the mutations (HARRIS *et al.* 1994; FOSTER and TRIMARCHI 1995; FOSTER *et al.* 1995). Recombinational double-strand break (DSB) repair in *Saccharomyces cerevisiae* has recently been shown to be accompanied by a high rate of mutation in a nearby gene (STRATHERN *et al.* 1995). To further elucidate how recombination is involved in adaptive mutation in FC40, we have investigated the role(s) of the enzymes that interact with recombination intermediates.

During recombination initiated by RecA-mediated strand exchange, four-way (Holliday) junctions are translocated by junction-specific helicases (branch migration). *E. coli* has two such enzymes, RuvAB and RecG, which appear to have overlapping functions (reviewed in WEST 1994). RuvC, a junction-specific endonuclease (resolvase) (CONNOLLY *et al.* 1991; IWASAKI *et al.* 1991), appears to act in concert with RuvAB (MANDAL *et al.* 1993; SHAH *et al.* 1994). It is not clear that RecG is associated with any resolvase activity, and RecG may promote the resolution of both three- and four-stranded junctions by a different mechanism (WHITBY *et al.* 1993). If adaptive mutations are produced or preserved by recombination, one or more of these enzymes could be involved.

In the study reported here, we tested the roles of

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RuvAB, RuvC, and RecG in adaptive reversion to Lac$^+$ in FC40. We found that RuvAB and RuvC are required for postplating mutation to Lac$^+$ but that RecG is not. Surprisingly, RecG appears to inhibit postplating mutations. These results have been assimilated into a model proposing that the collapse of a replication fork at a DNA nick is the initiating event for recombination and associated mutation (KUZMINOV 1995). According to this model, the most important conjugal function required for mutagenesis is nicking at *oriT*, and the continual creation of this nick produces the high rate of reversion of the *lac* allele on the episome.

MATERIALS AND METHODS

Bacterial strains and plasmids: The strains used are derivatives of P90C [F$^-$*ara* Δ(*lac-proB*)$_{XIII}$ *thi*] (COULONDRE and MILLER 1977) or FC36, a rifampicin-resistant (Rif^R) isolate of P90C (CAIRNS and FOSTER 1991). The Rif^R revertible strain, FC40, and the Rif^S nonrevertible scavenger, FC29, have been previously described in CAIRNS and FOSTER (1991). Additional strains used in this study (and references to the alleles) are listed in Table 1; all are isogenic to FC40 except where noted. Derivatives were constructed by first moving chromosomal alleles into the F$^-$ background by P1 bacteriophage-mediated transduction; alleles conferring severe recombination deficiencies, such a Δ(*recA*) and *recB21*, were introduced last. The *lacI33-lacZ proAB*$^+$ episome was then mated into the various backgrounds by selecting for proline prototrophy. Finally, if required, the episome-carrying strains were transformed with plasmid DNA. Standard techniques were used (MILLER 1972; AUSUBEL *et al.* 1991).

To make a *umuC36* derivative, P90C was transduced to *purB58 fadR613::*Tn*10*, then transduced to adenine prototrophy with a P1 lysate of a *purB*$^+$ *umuC36 fadR*$^+$ strain. Isolates were screened for tetracycline-sensitivity (Tet^S), and their nonmutability was confirmed by a lack of UV-induced mutation to Rif^R. *mutL::*Tn*10* was transduced by selection for Tet^R; the mutator phenotype was confirmed by increased reversion of F′ *lacI33-lacZ* after introducing the episome. The *ruv* and *recG* alleles were transduced by selecting for the appropriate drug resistances in the genes (*ruvA60::*Tn*10*, *ruvA59::*Tn*10*, Δ*ruvC64::*Kan, *recG258::*dTn*10*Kan, Δ*recG263::*Kan) or in nearby regions (*eda51::*Tn*10* for *ruv* genes; *zib636::*Tn*10* for *recG*; these insertions were confirmed to have no relevant phenotypes of their own). *zib636::*Tn*10* was transduced from strain RK4954 (KADNER and SHATTUCK-EIDENS 1983) into strain PC0297 (= *recG162*) (STORM *et al.* 1971) to make strain RM4703 (= *recG162 zib636::*Tn*10*). P1 grown on this strain was use to transduce MG1655 to *recG162 zib636::*Tn*10*, yielding strain RM4714, which served as donor to FC36 and its derivatives. All transductants were screened for their UV sensitivity, which is moderately increased in *recG* and *ruv* mutants and greatly increased in *recG ruv* double mutants (LLOYD 1991). Using transductional crosses, *recG258 ruvA60* and *recG258 ruvC53* strains were confirmed to have a 100-fold or greater reduction in recombination relative to wild type and each single mutant strain, as previously reported (LLOYD 1991). Δ(*recA*) *srl::*Tn*10* was transduced by selection for Tet^R and the UV-sensitive phenotype was confirmed. *recB21* was transduced by selection for a linked *argA::*Tn*10*; a spontaneous arginine prototroph of a UV-sensitive transductant was then isolated and confirmed to be Tet^S. The UV sensitivity of each construction was confirmed after mating in the episome, and subcultures were often retested when they were used for mutagenesis experiments.

Although *recG ruvC* double mutants could be created in the F$^-$ background, we found it difficult to maintain these strains during subsequent manipulations in minimal medium. Typically, after mating in the episome, mutation rates would vary among exconjugates. In the case of *recG258 ruvC53*, exconjugates with high mutation rates also had increased UV resistance, and we confirmed with back-crosses that the *ruvC53* allele had reverted. In the case of *recG162* Δ(*ruvC*)*64*, all four exconjugates tested were UV-sensitive, but their mutation rates varied (Table 2). Two isolates had moderate mutation rates at 37°, but these approached the level of *recG*$^-$ *ruv*$^+$ strains when the lactose plates were incubated at 30°. Two isolates had the low mutation rate of *recG ruvAB* strains (at both 37 and 30°), but when one of these isolates was subcultured in minimal medium for a large-scale test, its mutation rate appeared to have increased. As the Δ(*ruvC*)*64* allele should not be able to revert, these results suggest that suppressors of the *ruvC* defect can appear in the population, and may be temperature sensitive. In contrast, all but one of the *recG ruvA* and *recG ruvB* double-mutant strains were stable. The exception was *recG258 ruvB52*, which, when assayed for Lac$^+$ reversion, had the same mutation rate and UV sensitivity as *recG258*, indicating that the *ruvB52* allele had probably reverted.

*eda51::*Tn*10* was replaced with *eda51::*Tet*::*Cam using an allele made by cloning the gene for chloramphenicol resistance, *cat*$^+$, in the *Eco*RV site early in the *tetA* gene of Tn*10*. This insertion allele was then transferred into a F′ *lac::*Tet to make F′ *lac::*Tet*::*Cam (strain RM4353) using a phagemid-based allele replacement system (SLATER and MAURER 1993). P1 grown on RM4353 was used to transduce RM4672 (= MG1655 but *ruv*$^+$ *eda51::*Tn*10*) to Cam^R on minimal lactose medium. A Tet^S transductant contained the desired replacement (*eda51::*Tet*::*Cam; strain RM5268). P1 grown on RM5268 was used to donate *ruv*$^+$ to *ruvA60::*Tn*10* recipients. The *eda51::*Tet*::Cam* insertion was confirmed to have no relevant phenotype of its own.

pMQ315 is pBR322 carrying *mutS*$^+$, pMQ339 is pACYC184 carrying *mutL*$^+$ (WU and MARINUS 1994), pMQ133 is pBR322 carrying *dam*$^+$ (ARRAJ *et al.* 1990), and pBL125 is pBR322 carrying *recG*$^+$ (LLOYD and BUCKMAN 1991). Strains were transformed with these plasmids by selecting for the appropriate drug resistances; control strains and the scavenger strain were transformed with the vectors.

Postplating mutation rates: Postplating (time-dependent) mutation rates were determined as previously described (CAIRNS and FOSTER 1991; FOSTER 1994). Strains with F′ *lacI33-lacZ* were grown to saturation at 37° in M9 minimal medium (MILLER 1972) with 0.1% glycerol and appropriate drugs (22.5 mg/l kanamycin, 10 mg/l tetracycline, 17 mg/l chloramphenicol, or 50 mg/l carbenicillin). These cultures were diluted 10^5-fold into fresh medium, and four to 10 1-ml aliquots allowed to again reach saturation. The subcultures were then plated with 10^9 FC29 scavenger cells (grown in M9 0.1% glycerol) in 2.5 ml top agar (1% agar, 0.5% NaCl) on M9 plates containing 0.1% lactose. (FC29, which serves to utilize any contaminating carbon sources, can neither revert nor recombine with *lacI33-lacZ* to give a Lac$^+$ phenotype; CAIRNS and FOSTER 1991). Plates were incubated at 37°. For plasmid-bearing strains, the M9 lactose plates contained the appropriate drugs and the scavengers carried the vector.

For most experiments, the number of revertible cells plated was controlled by the amount of glycerol in the medium (0.1, 0.01, or 0.001%) and the final volume plated (1 or 0.1 ml). With the *mutL::*Tn*10* strains (Table 5), a slightly different

TABLE 1

The *Escherichia coli* strains used in this study

Strain	*ruv* allele	*recG* allele	Other alleles	Other drug markers	Donor strain	Obtained from	References to strains or alleles
FC40	+	+					CAIRNS and FOSTER (1991)
FC46[a]	+	+	*mutL*::Tn*10*		CM125	E. EISENSTADT	SIEGEL *et al.* (1982)
FC230[b]	+	+	*umuC36*		TK610	G. WALKER	KATO and SHINOURA (1977)
FC348	+	+	Δ(*recA*)	*srl301*::Tn*10*	JC10289	A. J. CLARK	CSONKA and CLARK (1979)
FC433/485/571[c]	*ruvA60*::Tn*10*	+			N2057	A. KUZMINOV, CGSE[d]	SHURVINTON *et al.* (1984)
FC438	+	*recG162*		*zib636*::Tn*10*	RM4714		This paper
FC459	+	*recG162*	*umuC36*	*zib636*::Tn*10*			
FC465/538	+	*recG258*::dTn*10*Kan			N2731	S. LOVETT	LLOYD and BUCKMAN (1991)
FC471/521/577[ce]	*ruvA60*::Tn*10*	*recG258*::dTn*10*Kan					
FC482[c]	*ruvC51*	+		*eda51*::Tn*10*	TNM554	T. KOGOMA	SHURVINTON *et al.* (1984)
FC483[c]	*ruvC53*	+		*eda51*::Tn*10*	CS85	T. KOGOMA	SHURVINTON *et al.* (1984)
FC526	+	Δ*recG263*::Kan			N3793	R. LLOYD	KALMAN *et al.* (1992), MANDAL *et al.* (1993)
FC527	+	*recG258*::dTn*10*Kan	Δ(*recA*)	*srl301*::Tn*10*			
FC536	+	*recG258*::dTn*10*Kan	*mutL*::Tn*10*				
FC539	+	*recG258*::dTn*10*Kan	*recB21*		SMR1	F. STAHL	WILLETTS *et al.* (1969)
FC567	*ruvB4*	+		*eda51*::Tn*10*	N1670	F. STAHL	OTSUJU *et al.* (1974)
FC568	*ruvB52*	+		*eda51*::Tn*10*	FB154	F. STAHL	SHURVINTON *et al.* (1984)
FC569	*ruvB54*	+		*eda51*::Tn*10*	FB155	F. STAHL	SHURVINTON *et al.* (1984)
FC570	*ruvA59*::Tn*10*	+			FB156	F. STAHL	SHURVINTON *et al.* (1984)
FC573	Δ(*ruvA-ruvC*)*65*	+			AM887	F. STAHL	MANDAL *et al.* (1993)
FC574	*ruvB4*	*recG258*::dTn*10*Kan		*eda51*::Tn*10*			
FC575	*ruvB54*	*recG258*::dTn*10*Kan		*eda51*::Tn*10*			
FC576	*ruvA59*::Tn*10*	*recG258*::dTn*10*Kan					
FC579/580[e]	*ruvA60*::Tn*10*	Δ*recG263*::Kan					
FC581	Δ(*ruvC*)*64*::Kan	+			GS1481	A. KUZMINOV	MANDAL *et al.* (1993)
FC582	Δ(*ruvC*)*64*::Kan	*recG162*		*zib636*::Tn*10*			
FC583	+	+		*eda51*::Tet::Cam	RM5268		This paper
FC584	+	*recG258*::dTn*10*Kan		*eda51*::Tet::Cam			
FC585	*ruvA60*→*ruvA*$^+$	+		*eda51*::Tet::Cam			
FC586	*ruvA60*→*ruvA*$^+$	*recG258*::dTn*10*Kan		*eda51*::Tet::Cam			

Strain	Episomal *tra* allele	Chromosomal *recG* allele	Other drug markers	Donor strain	Obtained from	References to alleles
FC396	*tra*$^+$	+	*zaj-3099*::Tn *10d*Kan	CAG18594	C. GROSS	SINGER *et al.* (1989)
FCJ65	*traD411*::Kan	+		pKI256	K. IPPEN-IHLER	K. IPPEN-IHLER (personal communication)
FCJ103	*traD411*::Kan	*recG162*	*zib636*::Tn *10*			

Strain	Plasmid	Chromosomal *recG* allele	Plasmid drug marker	Obtained from	References to plasmids
FC359	pMQ315, pMQ339	+	Ap, Cam	M. MARINUS	WU and MARINUS (1994)
FC370	pBR322, pACYC184	+	Ap, Cam		
FC377	pBR322	+	Ap		
FC469	pMQ133	+	Cam	M. MARINUS	ARRAJ *et al.* (1990)
FC473	pBR322	*recG258*::dTn*10*Kan	Ap		
FC479	pMQ133	*recG258*::dTn*10*Kan	Cam		
FC481	pBR322, pACYC184	*recG258*::dTn*10*Kan	Ap, Cam		
FC480	pMQ315, pMQ339	*recG258*::dTn*10*Kan	Ap, Cam		
FC497	pBL125	*recG258*::dTn*10*Kan	Ap	R. LLOYD	LLOYD and BUCKMAN (1991)
FC500	pBL125	+	Ap		
FC501	pBL125	*recG162*	Ap		

[a] A Rif^R isolate of the strain was obtained after construction.
[b] The strain is Rif^S.
[c] The strain was constructed from donors obtained from different laboratories, or after passing the allele through MG1655.
[d] *E. coli* Genetic Stock Center.
[e] The strain was constructed in each direction.

protocol was used to make these experiments strictly comparable to our previous ones with *mutS* strains (FOSTER and CAIRNS 1992). In this case, dilutions of the revertible cells were inoculated into M9 0.1% glycerol medium together with a predetermined number of FC29 cells so that at saturation, each culture would contain 10^6 to 10^7 cells of the revertible strain and 10^9 scavenger cells. Both methods accomplished the desired result of limiting the number of revertible cells so that, after plating, only 10 to 50 new Lac^+ colonies would appear each day from day 3 through day 5.

Before the cells were plated on lactose medium, their numbers were determined by plating dilutions on LB plates (MILLER 1972) with the appropriate drugs (45 mg/l kanamycin, 20 mg/l tetracycline, 34 mg/l chloramphenicol, 100 mg/l rifampicin, or 100 mg/l carbenicillin). In the case of *recG258*, *ruvA60*, *ruvC53*, and *recG258 ruvA60* strains, the cell numbers during incubation on lactose plates were determined by taking plugs from the plates as previously described (CAIRNS and FOSTER 1991). Neither loss of viability nor cell proliferation was detected. Because we were concerned that maintenance of the episome might be compromised in the *recG258 ruvA60* strain, we checked that cells taken from lactose plates after four days of incubation would grow on minimal glycerol-rifampicin plates, and therefore were Pro^+ (*i.e.*, they still retained their episomes). Twelve isolates of this strain taken from the lactose plates were confirmed to have the same UV sensitivity as the original *recG258 ruvA60* strain.

The postplating mutation rates to Lac^+ given in Tables 3 and 5 were calculated as the mean number of Lac^+ colonies appearing each day from days 3–5, normalized to 10^8 cells plated, and the error given is the standard error of the mean (SE).

Semiquantitative assays of postplating mutation rates: To screen a large number of strains rapidly, we utilized a semiquantitative assay (CAIRNS and FOSTER 1991). Aliquots (10 μl) of saturated cultures grown in M9-0.1% glycerol were spread on each quadrant of an M9-lactose plate. Each assay consisted of four or more independent cultures of the strain to be tested plus appropriate controls. Plates were incubated at 37°, and Lac^+ colonies were counted daily starting on day 2. Because these tests involve relatively few cells ($\sim 10^7$ cells per quadrant), no scavengers are used to utilize contaminating energy sources, and each population is subject to cross-feeding by Lac^+ colonies on other quadrants, the total cell number increases about 10-fold over the duration of the test. Such growth tends to obscure small differences in mutation rates among strains. But, when these differences are large, we have found that the semiquantitative test is a reliable indicator of results obtained with the usual large-scale experiment.

With these assays, postplating mutation rates to Lac^+ were estimated as the mean number of Lac^+ colonies appearing per quadrant each day from days 3–5. Although the accumulation of Lac^+ mutants was often not linear in these assays, the mean still was a reasonable way to summarize the data, and also allowed an error to be estimated (the SE). These results are in Table 2.

Generation-dependent mutation rates: Generation-dependent (preplating) rates of reversion to Lac^+ were determined in wild-type and *recG258* cells for F′ *lacI33-lacZ*, and for the *lacI33-lacZ* allele on the chromosome. Large fluctuation tests (40–50 independent cultures) were done as described above, but only early-arising Lac^+ colonies (those appearing by day 2 for the cells with the episomal allele and by day 3 for cells with the chromosomal allele) were counted. The number of cells in each culture was limited by the glycerol concentration and was chosen so that when the entire culture was plated, $\geq$50% of the lactose plates would have no Lac^+ colonies by day 2 or by day 3. Mutations per culture, *m*, was calculated from the proportion of cultures with no mutants (LEA and COULSON 1949). Mutations per cell, *M*, was calculated as $m/(2N)$, where N is the number of cells plated. To determine the generation-dependent mutation rates to Rif^R, Nal^R (resistance to nalidixic acid) and $Strep^R$ (resistance to streptomycin), 16 1-ml LB cultures of P90C and its *recG258* derivative (PF1969) were grown from 10^3 cells to saturation, and 0.1 ml aliquots were plated on LB plates containing the drugs (100 mg/l rifampicin, 40 mg/l nalidixic acid, or 200 mg/l streptomycin). For mutation to Nal^R and $Strep^R$, the proportion of zeros was again used to calculate mutation rates. Mutation rates to Rif^R were calculated by the maximal likelihood method (LEA and COULSON 1949). Because of phenotypic delay, these rates may be underestimates, but we are concerned here only with comparing the rates among the strains. These results are in Table 4.

Quantitative mating assays: The protocol described in KATHIR and IPPEN-IHLER (1991) was used to determine the efficiencies with which wild-type and *recG258* cells transfer F′ *lacI33-lacZ* and its *traD411* derivative. The Rif^R $Strep^S$ F′(*lacI33-lacZ*$proAB^+$) donors and a Rif^S $Strep^R$ F^- Pro^- recipient (FC599) were grown to midexponential phase ($\sim 10^8$ cells per ml) in LB broth at 37° with gentle agitation. Donors and recipients were mixed in triplicate at a 1:4 ratio and incubation was continued for 40 min. The mating mixtures were then centrifuged, resuspended in saline and vigorously vortexed, and appropriate dilutions were plated on LB-rifampicin (to titer the donors), LB-streptomycin (to titer the recipients), and Volgel-Bonner minimal glucose medium (VOGEL and BONNER 1956) plus streptomycin (to titer Pro^+ cells among the recipients). Donors and recipients were also plated separately on minimal-streptomycin plates to determine the level of spontaneous mutation to $Strep^R$ and Pro^+. No $Strep^R$ cells were detected among the donors, but the recipient population contained about 40 Pro^+ mutants per 10^8 cells. Mating efficiencies (Table 3) were calculated as the mean number of Pro^+ episomes transferred per donor cell, $\pm$ the SE.

RESULTS

RecA-dependent adaptive reversion also depends on RuvAB and RuvC: As shown in Figure 1, a *ruvA* mutant, *ruvA60::*Tn*10*, and a *ruvC* mutant, *ruvC53*, each reduced by about 10-fold the rate of postplating reversion of F′ *lacI33-lacZ*. These results were confirmed in semiquantitative tests (see MATERIALS AND METHODS) with a second allele of *ruvA*, *ruvA59::*Tn*10* (which, like *ruvA60::*Tn*10*, is polar on *ruvB*, the second gene in the *ruvAB* operon) (SHARPLES *et al.* 1990). In addition, we tested three alleles of *ruvB*, namely *ruvB4*, *ruvB52*, and *ruvB54*, two additional alleles of *ruvC*, namely *ruvC51* and Δ*ruvC64::*Kan, and Δ(*ruvA-ruvC*)*65*, which has a deletion extending from *ruvC* (which is upstream of the *ruvAB* operon; SHARPLES *et al.* 1990; TAKAHAGI *et al.* 1991) to *ruvA*. In all cases, postplating reversion to Lac^+ was severely inhibited by the *ruv* defects (Table 2).

RecA-dependent adaptive reversion is enhanced by mutations in *recG*: When cells carried a mutation in *recG*, *recG258::*dTn*10*Kan, the rate of postplating Lac^+ mutation was enhanced 100-fold (Figure 2; note difference in scale compared to Figure 1). The same results

TABLE 2

Semiquantitative estimates of reversion rates of F′ *lacI33-lacZ* in various genetic backgrounds

Strain	Relevant genotype	n^a	Day 2 (Lac^+/sector)[b]	Days 3–5 (Lac^+/sector/day)[c]
Wild type				
FC40	+	22	3 ± 1	36 ± 2[d]
ruv strains				
FC433/485/571	*ruvA60*	16	0	6 ± 1[d]
FC570	*ruvA59*	4	0	4 ± 2
FC567	*ruvB4*	4	0	10 ± 5
FC568	*ruvB52*	4	2 ± 2	5 ± 2
FC569	*ruvB54*	4	0	8 ± 3
FC482	*ruvC51*	3	0	3 ± 1
FC483	*ruvC53*	4	0	2 ± 1
FC581	Δ(*ruvC*)*64*	4	0	1 ± 1[d]
FC573	Δ(*ruvA-ruvC*)*65*	4	0	6 ± 2
recG strains				
FC465/538	*recG258*	8	10 ± 1	177 ± 14[d]
FC438	*recG162*	6	5 ± 1	186 ± 13
FC526	Δ*recG263*	2	15, 23	251, 258
recG ruv strains				
FC471/521/577	*recG258 ruvA60*	12	0	1 ± 1[d]
FC576	*recG258 ruvA59*	4	0	1 ± 1
FC574	*recG258 ruvB4*	4	0	3 ± 2
FC575	*recG258 ruvB54*	4	0	1 ± 1
FC579/580	Δ*recG263 ruvA60*	8	0	3 ± 1
FC582[e]	*recG162* Δ(*ruvC*)*64*	4	0	3 ± 1
FC582[e]	*recG162* Δ(*ruvC*)*64*	4	2 ± 2	22 ± 7
Other strains				
FC348	Δ(*recA*)	4	0	1 ± 0.3
FC527	*recG258* Δ(*recA*)	4	0	1 ± 0.1
FC404	*recB21*	4	0	1 ± 0.2[d]
FC539	*recG258 recB21*	4	0	14 ± 4[d]

[a] Number of independent cultures tested.
[b] Mean number of Lac^+ colonies appearing on day 2, ± the SE where appropriate.
[c] Mean number of Lac^+ colonies appearing each day for as long as the colonies could be counted, ± the SE where appropriate.
[d] These results were confirmed in large-scale quantitative experiments (see MATERIALS AND METHODS).
[e] Two isolates had low mutation rates and two had higher mutation rates; each were tested twice (see RESULTS).

were obtained with two additional alleles of *recG*, namely *recG162* and Δ*recG263*::Kan (Table 2). In addition, supplying excess $RecG^+$ from a plasmid more than complemented the mutator phenotype of *recG258* (Figures 2 and 3) and *recG162* (data not shown), reducing the mutation rate to about one-fifth the rate observed in FC40. Overproduction of $RecG^+$ in the wild-type strain had a similar effect (Figure 3).

The postplating mutations appearing in *recG* mutant cells share many of the features of those appearing in wild-type cells. They depend completely on *recA*$^+$ and substantially on *recB*$^+$ (Table 2), indicating that they require recombination functions. However, they are not affected by a defect in *umuC*, which is required for SOS-promoted error-prone DNA synthesis (data not shown). They depend substantially on the conjugal function supplied by *traD*$^+$ (Table 3). Their mutational spectrum also resembles that of mutations in wild-type cells: using oligonucleotide probing (FOSTER *et al.* 1995), we determined that 31/45 Lac^+ mutations arising on days 3–5 in the *recG258* background were −1-bp deletions at the major hotspot for postplating mutations (FOSTER and TRIMARCHI 1994; ROSENBERG *et al.* 1994). In addition, as with wild-type cells, Lac^+ mutations do not appear when *recG* mutant cells are starved in the absence of lactose (data not shown).

The loss of RecG does not confer a general mutator phenotype: In *recG* mutants the number of Lac^+ colonies appearing 2 days after plating was up to 10-fold higher than in FC40. Early-appearing colonies are generally taken to be due to preplating (generation-dependent) mutations (CAIRNS and FOSTER 1991), but this assumption is unlikely to be valid in a strain that has a high rate of postplating mutation. Therefore, to check whether *recG* defects affect generation-dependent mutation rates, we used lethal selections that do not allow postplating mutation. Mutation rates to Nal^R (mutations in *gyrA*), Rif^R (mutations in *rpoB*), and $Strep^R$ (mu-

TABLE 3

The effect of a defect in *traD* on reversion of F′ *lacI33-lacZ* and on mating efficiencies

			Reversion to Lac^{+a}			Mating efficiency	
Strain	*recG* allele	Conjugal genotype	n^b	Day 2 (Lac$^+$/10^8 cells plated)^c	Days 3–5 (Lac$^+$/day/10^8 cells plated)^d	n^b	Pro$^+$ transferred per donor cell
FC396/40^e	Wild type	*tra*$^+$	10	4 ± 1	19 ± 1	3	0.6 ± 0.1
FCJ65^e	Wild type	*traD411*::Kan	5	1 ± 1	2 ± 0.4	3	<1 × 10^{-6}
FC438	*recG162*	*tra*$^+$	5	0	965 ± 142	3	0.9 ± 0.1
FCJ103	*recG162*	*traD411*::Kan	5	0	214 ± 50	3	<1 × 10^{-6}

[a] Results are from large-scale quantitative experiments.
[b] Number of independent cultures in the experiment.
[c] The mean number of Lac$^+$ colonies appearing on day 2, normalized to 10^8 cells plated, ± the SE where appropriate.
[d] The mean number of Lac$^+$ colonies appearing each day, normalized to 10^8 cells plated, ± the SE.
[e] For comparison, Lac$^+$ reversion rates of FC396 and FCJ65 from Figure 1 of Foster and Trimarchi (1995) are given; FC396 is FC40 but *zaj-3099*::Tn*10*dKan on the episome (which has no relevant phenotype); FCJ65 is FC40 but *traD411*::Kan. FC40 and FCJ65 were used in the mating assay.

tations in *rpsL*) (Vinopal 1987) were determined in the Rif^S parent of FC40, P90C, and its *recG258* derivative. In addition, we compared reversion rates of the *lacI33-lacZ* allele on the chromosome (where it does not undergo RecA-dependent adaptive mutation; Foster and Trimarchi 1995; Galitski and Roth 1995) in wild-type and *recG* mutant strains. There was little indication that *recG* mutant cells had an elevated rate for any of these chromosomal mutations (Table 4).

RuvAB is required for enhanced adaptive mutation in the *recG*$^-$ background: As shown in Figure 4, the *ruvA60* allele completely eliminated the increase in postplating Lac$^+$ mutations due to *recG258*. The same result was obtained with *ruvA59*, *ruvB4*, and *ruvB54* in *recG258* cells, and with a Δ*recG263 ruvA60* strain (Table 2). To confirm that this inhibition was due to loss of *ruvAB*, and not due to the gain of a suppressor of *recG*$^-$, we transduced the *recG258 ruvA60* double mutant to *ruvA*$^+$, and the mutation rate returned to the high level seen in the original *recG* mutant strains (Figure 5).

Because of the genetic instability of *recG ruvC* double mutant strains (see Materials and Methods), it is difficult to say if RuvC is also required for enhanced adaptive mutation in RecG$^-$ cells. The mutation rates of four

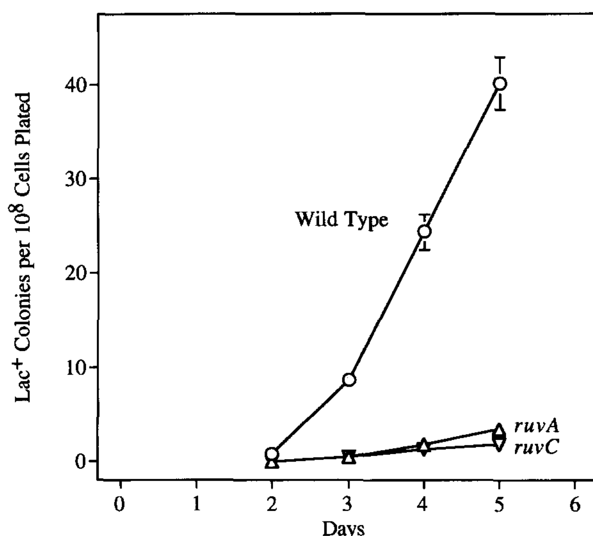

Figure 1.—Inhibition of postplating reversion of F′ *lacI33-lacZ* by defects in *ruvA* and *ruvC*. ○, FC40 (wild type); △, FC433 (*ruvA60*); ▽, FC483 (*ruvC53*). Two experiments are shown, with the results for FC40 combined. 2 × 10^8 cells from each of 10 independent cultures of FC433 and FC483, and 14 independent cultures of FC40, were plated with 2 × 10^9 FC29 scavenger cells. Points are means and error bars are ± SEs (some of which are smaller than the symbols).

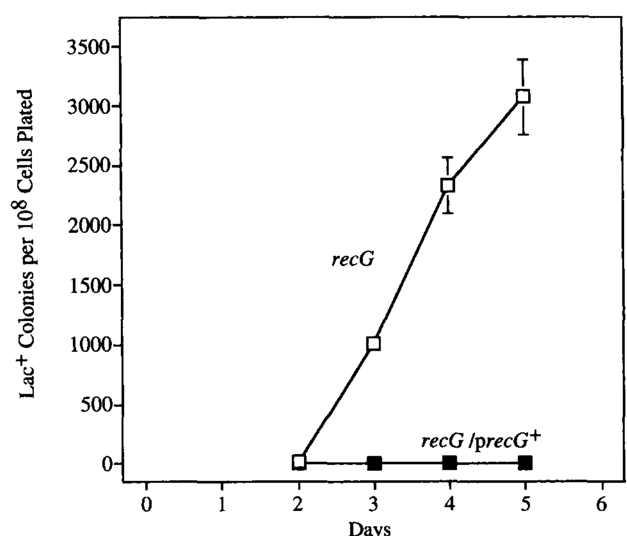

Figure 2.—Stimulation of postplating reversion of F′ *lacI33-lacZ* by a *recG* mutant and the complementation of this phenotype by a RecG$^+$ plasmid. □, FC465 (*recG258*); ■, FC497 (*recG258*/p*recG*$^+$). 2 × 10^8 cells of FC497 and 4 × 10^6 cells of FC465 from each of five independent cultures were plated with 2 × 10^9 FC29 scavenger cells or FC29 carrying pBR322. Points are means and error bars are ± SEs (some of which are smaller than the symbols).

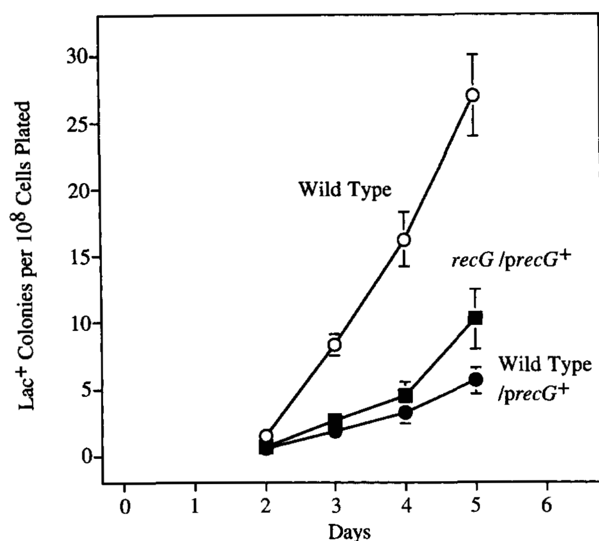

FIGURE 3.—Overproduction of RecG$^+$ inhibits postplating reversion of F′ *lacI33-lacZ* in wild-type cells. ○, FC40 (wild type); ●, FC500 (wild type/p*recG*$^+$); ■, FC497 (recG258/p*recG*$^+$). This is the same experiment as shown in Figure 2, with the addition of the data for FC40 and FC500. 2×10^8 cells of FC40 and FC500 from each of five independent cultures were plated with 2×10^9 FC29 scavenger cells or FC29 carrying pBR322. Points are means and error bars are ± SEs (some of which are smaller than the symbols).

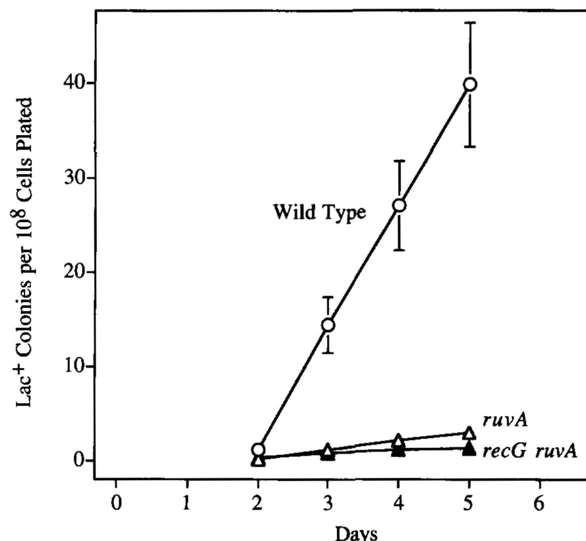

FIGURE 4.—RuvA$^+$ is required for the stimulation of postplating mutation in *recG* mutant cells. ○, FC40 (wild type); △, FC485 (*ruvA60*); ▲, FC521 (*recG258 ruvA60*). 2×10^8 cells of each strain from each of five independent cultures were plated with 2×10^9 FC29 scavenger cells. Points are means and error bars are ± SEs (some of which are smaller than the symbols).

isolates obtained after mating the F′ *lacI33-lacZ* episome into a *recG162* Δ(*ruvC*)*64* strain are shown in Table 2. Two isolates had the low mutation rate of *recG ruvAB* double mutant strains and two isolates had somewhat higher rates, although still well below *recG* mutant levels at 37°. Thus, we suspect that RuvC is required for most, if not all, of the postplating mutations in *recG*$^-$ cells, but that suppressors of the *ruvC* defect arise in the population of *recG ruvC* double-mutant cells.

Interaction between the *recG*$^-$ phenotype and methyl-directed mismatch repair: Inactivating the MutH,L,S methyl-directed mismatch repair system (MMR) (see FRIEDBERG *et al.* 1995 for a review) has as great an effect on postplating mutation in FC40 as does inactivating *recG* (FOSTER and CAIRNS 1992). To determine whether RecG and MMR independently affect postplating mutation rates, we tested the phenotype of *recG258* under three conditions of altered MMR activity (Table 5). First, a defect in *mutL*, which inactivates MMR, increased the postplating mutation rate 100-fold in wild-type cells, but only threefold in the *recG* mutant. Second, reducing MMR activity by supplying the Dam methylase on a multicopy plasmid increased postplating mutations 10-fold in FC40, but had no effect on post-

TABLE 4

Generation-dependent mutation rates in wild-type and *recG* mutant cells

	Resistance to			Reversion of *lacI33-lacZ*	
Strain	Rifampicin[a]	Nalidixic acid[b]	Streptomycin[c]	Chromosomal[d]	Episomal[e]
Wild type	17	1.8	0.4	0.13	4.1
recG258	12	1.0	1.7	0.12	37

Mutations are per 10^9 cells as determined in fluctuation tests (see MATERIALS AND METHODS).

[a] The mean number of Rif^R mutants per 10^8 cells plated (± the SE) was 6.8 ± 0.6 for the wild-type strain (leaving out one culture with 268 mutants) and 5.2 ± 0.8 for the *recG258* strain.

[b] The mean number of Nal^R mutants per 10^8 cells plated (± the SE) was 0.5 ± 0.2 for the wild-type strain (leaving out two cultures with 11 and 29 mutants, respectively) and 0.4 ± 0.2 for the *recG258* strain.

[c] The mean number of Strep^R mutants per 10^8 cells plated (± the SE) was 0.1 ± 0.1 for the wild-type strain and 0.5 ± 0.1 for the *recG258* strain.

[d] The mean number of Lac$^+$ mutants per 10^9 cells plated (± the SE) was 0.2 ± 0.1 for the wild-type strain (leaving out two cultures with 6 and 12 mutants, respectively) and 0.2 ± 0.1 for the *recG258* strain (leaving out one culture with 64 mutants).

[e] The mean number of Lac$^+$ mutants per 10^7 cells plated (± the SE) was 0.3 ± 0.1 for the wild-type strain and 0.9 ± 0.1 for the *recG258* strain (leaving out two cultures with 11 and 41 mutants respectively).

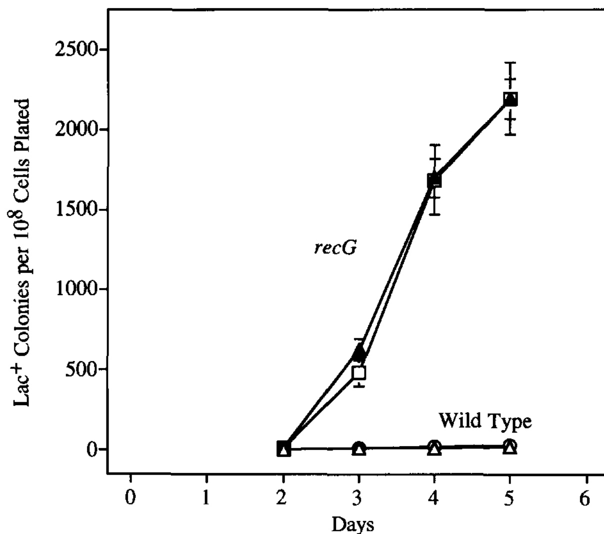

FIGURE 5.—Transducing a *recG ruvA* double mutant to $ruvA^+$ restores postplating mutation to the level of *recG* mutant cells. ○, FC583 (wild type but *eda*::Cam::Tet); □, FC584 (*recG258* but *eda*::Cam::Tet); △, FC585 (*ruvA60*::Tn*10* transduced to $ruvA^+$ *eda*::Cam::Tet); ▲, FC586 (*recG258 ruvA60*::Tn*10* transduced to $ruvA^+$ *eda*::Cam::Tet). 2×10^8 cells of FC583 and FC585 and 2×10^6 cells of FC584 and FC586 from each of five independent cultures were plated with 2×10^9 FC29 scavenger cells. Two experiments are shown, one with FC583 and FC585 and one with FC584 and FC586. Points are means and error bars are ± SEs (some of which are smaller than the symbols).

plating mutations in the *recG* mutant. Excess Dam overmethylates the *dam* methylation sites in the DNA, which interferes with strand incision (MARINUS *et al.* 1984). Finally, joint overproduction of MutS and MutL, which should enhance MMR, decreased postplating mutations modestly in FC40 (FOSTER *et al.* 1995 and Table 5), but dramatically in the *recG* mutant (Table 5). These results show that postplating mutations, whether in wild type or in *recG* mutants are, in principle, susceptible to MMR. However, in wild-type cells, mutations appear to be well corrected by MMR because reduction of MMR has a large effect and increase in MMR has a small effect. Conversely, in *recG* mutant cells, mutations appear to be poorly corrected by MMR because reduction of MMR has little effect and increase of MMR has a dramatic effect. The latter result is so striking that it seems possible that the excess MMR proteins may directly interfere with the mutagenic process in *recG* mutant cells (see DISCUSSION).

DISCUSSION

Our results demonstrate that *E. coli*'s systems for translocating Holliday junctions, RuvAB and RecG, have opposite effects on the production of RecA-dependent adaptive mutations in FC40. RuvAB is required for these mutations, whereas RecG inhibits them. Indeed, the two systems appear to be in competition, because overproduction of RecG reduces mutations even in the presence of RuvAB (Figure 3). These results support evidence previously obtained *in vitro* that RecG and RuvAB are not strictly redundant, but have different interactions with recombination intermediates (WHITBY *et al.* 1993; WHITBY and LLOYD 1995).

Because the two systems appear to be in competition, it is tempting to conclude that all the mutations recovered in *recG* mutants are simply the ones that RecG

TABLE 5

Effects of alterations in mismatch repair capability on reversion of F′ *lacI33-lacZ* in wild-type and *recG* mutant cells

Strain	*recG* allele	MMR genotype	n^a	Day 2 ($Lac^+/10^8$ cells plated)[b]	Days 3–5 (Lac^+/day/10^8 cells plated)[c]
FC40[d]	Wild type	$mutL^+$	14	1 ± 0.2	13 ± 1
FC46	Wild type	*mutL*::Tn*10*	5	111 ± 37	1047 ± 106
FC465	*recG258*	$mutL^+$	5	6 ± 6	1538 ± 61
FC536	*recG258*	*mutL*::Tn*10*	3[e]	255 ± 47	3843 ± 194
FC377	Wild type	Vector	5	0.4 ± 0.3	8 ± 1
FC469	Wild type	Dam^+	5	2 ± 1	136 ± 22
FC473	*recG258*	Vector	5	0	1152 ± 127
FC479	*recG258*	Dam^+	5	33 ± 14	1222 ± 202
FC370	Wild type	Vectors	5	0.5 ± 0.1	10 ± 1
FC359	Wild type	MMR^+	5	1 ± 0.6	5 ± 1
FC481	*recG258*	Vectors	5	0	401 ± 81
FC480	*recG258*	MMR^+	5	0	1 ± 0.2

The results are from quantitative, large-scale experiments (see MATERIALS AND METHODS).
[a] Number of independent cultures in the experiment.
[b] The mean number of Lac^+ colonies appearing on day 2, normalized to 10^8 cells plated, ± the SE where appropriate.
[c] The mean number of Lac^+ colonies appearing each day, normalized to 10^8 cells plated, ± the SE.
[d] Data for the wild type are from Figure 1.
[e] Two cultures were eliminated because they contained jackpots.

would normally prevent in wild-type cells. However, there are hints in our results that the mutational process uncovered when RecG is absent is slightly different. In the *recG* mutant background, mutations were less dependent on RecBC, less dependent on TraD, and less well corrected by mismatch repair than were mutations in wild-type cells. In addition, it appeared that a greater proportion of the mutations recovered in the *recG* mutant background were at the strong -1 frameshift hotspot in *lacI33*. Interestingly, a similarly increased mutational specificity was seen when a proofreading-defective allele of DNA polymerase II was introduced into FC40 (FOSTER *et al.* 1995). These results suggest that when RecG is absent, not only is the RuvAB-pathway more active in producing or recovering mutations, but some additional mutational pathways are also more active. This will be further discussed below.

When multiply defective strains are created, there is always a danger that certain combinations may result in a strong selection for suppressors. To minimize this possibility, we constructed *recG*, *ruv*, and double-mutant strains with various alleles transduced from different donor strains. In the case of *recG* mutants, the mutator phenotype could be complemented by supplying RecG$^+$ from a plasmid (Figure 2), indicating that no other gene in the *recG* mutant background was responsible for the phenotype. The same experiment was not possible with *ruvAB* mutants because overproduction of RuvAB, even from a low-copy plasmid, was severely debilitating to the cells (SHARPLES *et al.* 1990; P. L. FOSTER, J. M. TRIMARCHI, and R. A. MAURER, unpublished results). However, when we transduced the *ruvA*$^+$ allele into the *recG ruvA* double mutant, the mutation rate returned to the RecG$^-$ level (Figure 5). Thus, if there is a suppressor of the *recG*$^-$ defect in the *recG ruvAB* double mutants, the suppressor must be closely linked to the *ruv* locus, it must exist in the various donor strains used or be created during each construction, and it must not suppress the UV-sensitive or recombination-defective phenotypes of *ruv recG* double mutants. We think this unlikely.

Although the simultaneous loss of both RecG and RuvAB was relatively well tolerated, the simultaneous loss of both RecG and RuvC compromised the ability of the cells to grow or survive, or to maintain their episomes, in minimal medium. Because two isolates of the *recG162* Δ(*ruvC*)*64* strain initially had the low mutation rates of *recG ruvAB* mutants (Table 2), we suspect that RuvC is required for most, if not all, postplating mutations in *recG* defective cells, but that, at least in the *recG* mutant background, the *ruvC*$^-$ defect can be suppressed.

Both *recA* and *ruvAB* are repressed by LexA, the common repressor of the genes of the SOS regulon (see FRIEDBERG *et al.* 1995 for a review). Previously, one of us (P. L. FOSTER) reported that postplating mutations in FC40 were reduced by a noninducible *lexA* allele (*lexA3*; MOUNT *et al.* 1972), but that a normal level of mutation could be restored by an operator-constitutive allele of *recA* (*recAo281*; VOLKERT *et al.* 1981) (CAIRNS and FOSTER 1991). Because of the requirement for RuvAB reported here, we reinvestigated this result and discovered that the supposed *recAo281 lexA3* strain, FC237, was not *lexA3*. With a reconstructed strain, derepression of RecA was not sufficient to completely overcome the antimutagenic phenotype of *lexA3*. We are currently investigating whether derepression of RuvAB is also required, or if there are additional LexA-repressed functions involved in adaptive mutation in FC40.

That adaptive mutation in FC40 depends on RecBCD activity implicates duplex DNA ends (HARRIS *et al.* 1994), the entry point for RecBCD exonuclease (reviewed in MYERS and STAHL 1994; KOWALCZYKOWSKI *et al.* 1994). It has been proposed that the RecBCD pathway for recombination is used mostly for repair of disintegrated replication forks (KUZMINOV *et al.* 1994; KUZMINOV 1995). According to this model, a replication fork disintegrates when it encounters a single-strand interruption in the template DNA, allowing entry of the RecBCD exonuclease into the duplex DNA of the broken arm to initiate recombinational repair. In F, the conjugal origin, *oriT*, is nicked by an F-specific protein, TraI, initiating replacement-strand DNA replication and transfer of the displaced strand (reviewed in FROST *et al.* 1994; WILKINS 1995). KUZMINOV (1995) hypothesized that ordinary replication forks disintegrate at this nick, and the resulting recombinational repair gives rise to mutations. Thus, the primary role of conjugal functions in producing RecA-dependent adaptive mutations in FC40 may be to promote efficient nicking at *oriT* in the stationary cells. This could explain why defects such as *traD411*, that virtually abolish conjugation, have a less severe effect on adaptive mutation (TABLE 3; FOSTER and TRIMARCHI 1995)

The model in Figure 6 is based on the one presented by KUZMINOV (1995). A replication fork is assumed to have originated at the unidirectional vegetative origin, *oriS* (WILLETTS and SKURRAY 1987), although a counterclockwise moving fork initiated at the bidirectional origin, *oriV*, could have the same result (Figure 6, A and B). The fork disintegrates when it meets the nick at *oriT* (Figure 6C). RecBCD loads onto the free arm, degrades the DNA past the *lac* region, and, acting as a helicase, provides the single-stranded substrates for RecA-promoted strand invasion (Figure 6D). (In the intramolecular reaction modeled here, the remaining 5′ end at the nick site is assumed to be eventually ligated to the 3′ end of the nascent DNA; this joining may require removal of the covalently attached TraI protein. In an intermolecular reaction, the free arm could invade another, unnicked, copy of F). After both strands

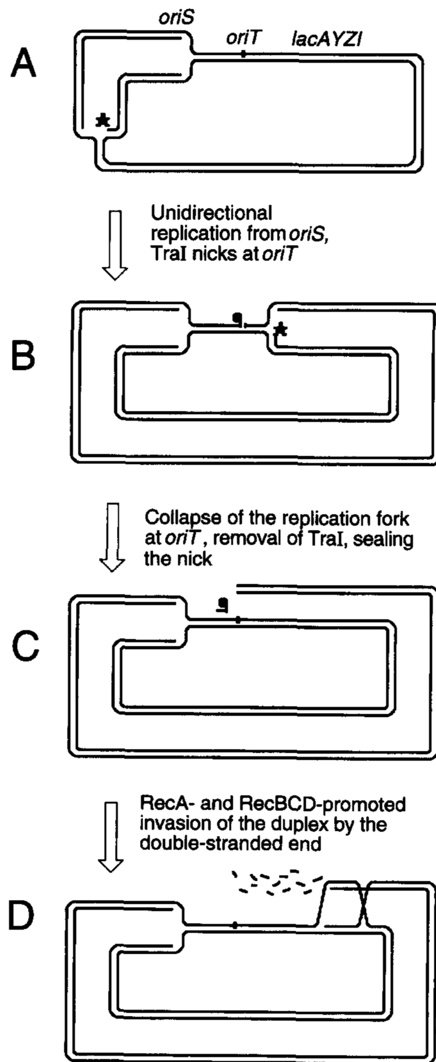

FIGURE 6.—Initiation of double-strand end invasion by collapse of the replication fork at *oriT*. A star marks the 3′ end of the counterclockwise moving fork. TraI is indicated by a flag.

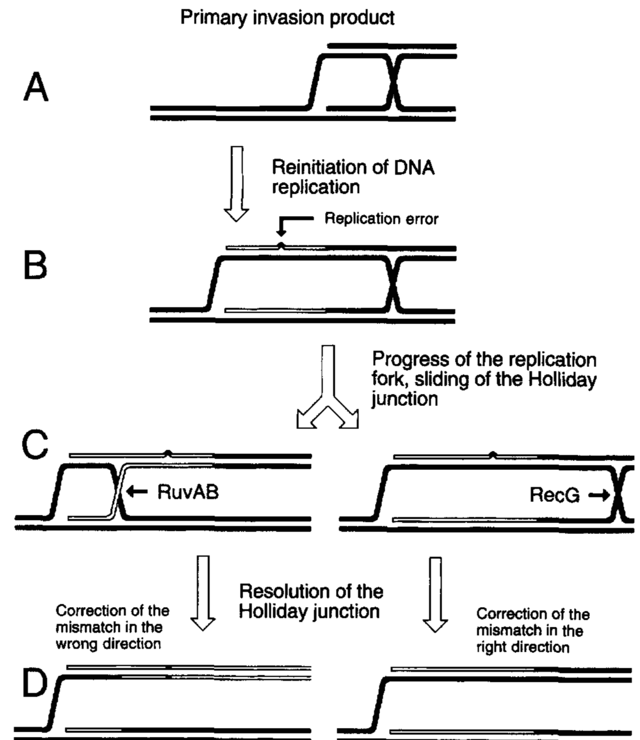

FIGURE 7.—Reestablishment of the replication fork and translocation of the Holliday junction in opposite directions by RuvAB and RecG. Newly synthesized DNA is indicated by open lines, template DNA by closed lines. The 3′ end is the lower strand in each case.

have invaded, a new replication fork is established (Figure 7A). However, this fork differs from a normal fork in that it is accompanied by a Holliday junction.

As the new replication fork traverses *lac* (Figure 7B), the fate of any replication errors will depend on the movement of the accompanying Holliday junction. If the Holliday junction is translocated in the direction of the new fork and also traverses *lac*, then the *lac* region will exist as conservatively replicated duplexes, one duplex containing two parental strands and the other two nascent strands (Figure 7C). The latter, being unmethylated, will be corrected at random by the MMR system, and replication errors will be as likely to be preserved as corrected (Figure 7D). In contrast, if the Holliday junction is translocated away from the fork, or does not traverse the newly synthesized DNA, then *lac* would exist as ordinary hemimethylated DNA, and sequence errors would be corrected.

In Figures 7 and 8, we present two ways that RuvAB and RecG could influence these events. *In vitro* evidence suggests that, in the presence of RecA, RuvAB and RecG translocate Holliday junctions in opposite directions (WHITBY *et al.* 1993). The polarity is established by RecA, which polymerizes 5′ to 3′ on single-stranded DNA (KOWALCZYKOWSKI *et al.* 1994). Assuming that the more invasive 3′ end initiates DNA synthesis and restoration of the replication fork, then the Holliday junction would be translocated toward the fork by RuvAB and away from the fork by RecG (Figure 7C). However, the *in vitro* activities of RuvAB and RecG are very sensitive to reaction conditions (WHITBY *et al.* 1993), so it is possible that, *in vivo*, they do not differ significantly in polarity. In Figure 8, it is assumed that RuvAB and RecG translocate the Holliday junction with the same polarity, which is determined by the binding of the RecA filament between the Holliday junction and the fork. Both RuvAB and RecG push the junction toward the fork, but RecG does so before DNA synthesis has initiated. The displaced strand is nicked (Figure 8B), ligated to the invading 3′ strand, and then RecG removes the Holliday junction (Figure 8C), allowing restoration of normal semiconservative replication (Figure 8D). Indeed, translocation with an associated nicking activity could be the alternative mechanism by which RecG removes Holliday junctions (A. KUZMINOV, personal com-

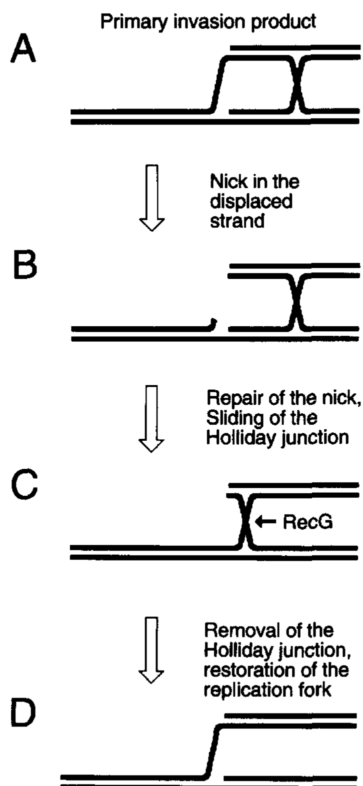

FIGURE 8. Removal of the Holliday junction by RecG and subsequent reestablishment of the replication fork. The 3′ end is the lower strand in each case.

munication). In both of these models (Figures 7 and 8), the RuvAB pathway is mutagenic and the RecG pathway acts in opposition to it.

Although we have proposed here that both RuvAB and RecG interact with the four-stranded Holliday junction, there is also evidence obtained *in vitro* that RecG binds and translocates three-stranded junctions (WHITBY and LLOYD 1995). In the case of 3′ invading ends, by moving the junction in reverse polarity to RecA, RecG would drive three-stranded junctions back toward duplex-duplex DNA, thereby helping to set up (four-stranded) Holliday junctions for RuvAB (WHITBY and LLOYD 1995). In the absence of RecG, junctions would also eventually be translocated into duplex-duplex DNA (spontaneously or by RecA), but at a slower rate. Thus, in *recG* mutants, 3′ ends may persist longer, increasing the amount of DNA synthesis (R. G. LLOYD, personal communication). D-loop-primed DNA synthesis has also been proposed to account for RecA-dependent "stable" DNA replication (ASAI and KOGOMA 1994). This extra synthesis, particularly if error-prone, could be contributing to the high level and somewhat abnormal features of adaptive mutation in the *recG* mutant strains. Nonetheless, our data indicate that preservation of these mutations would require establishment of the four-stranded substrate for RuvAB translocation.

In the general model, MMR promotes either the correction of errors or their retention, depending on the methylation status of the error-containing duplex DNA. If the Mut proteins gain access to the newly synthesized DNA before it is methylated and before RuvAB translocation (Figure 7B), mutations are prevented. But, after RuvAB translocation (Figure 7C), mismatches are corrected at random, preserving half the sequence errors. The model does not make clear predictions for the effect of MMR in the absence of RecG, which may depend on the relative speed of the various reactions. That decreases in MMR capacity had a relatively small effect in *recG* mutant cells (Table 5) supports the idea that, in the absence of RecG, RuvAB translocation is able to preserve more polymerase errors as mutations. However, the same results would be obtained if excess mismatches are produced in the *recG* mutant cells, and these partially saturate the cell's mismatch repair capacity. MMR has been found to saturate in the presence of other powerful mutators (DAMAGNEZ *et al.* 1989; SCHAAPER 1989), and to give rise to a high level of frameshift mutations (CUPPLES *et al.* 1990). It was surprising that overproduction of MutS and MutL prevented more mutations in *recG* mutant cells than in wild-type cells (Table 5). This suggests that RecG protects the DNA from MMR, or removes the Mut proteins from the DNA where they may conceivably inhibit RuvAB translocation. It is unclear if this activity of RecG against the Mut proteins would be important in cells with normal levels of the proteins.

The Kuzminov model of replication-fork collapse is attractive because of its simplicity and because it accounts for all the activities that are known to be required for RecA-dependent adaptive reversion in FC40. Other models require additional assumptions. For example, a similar sequence of events could be initiated by invasion of the linear DNA produced by conjugal replacement-strand synthesis, but, to provide a duplex end for RecBCD, lagging-strand synthesis would have to take place and the TraI protein would have to be removed from the 5′ end. However, our data do suggest that, at least in $RecG^-$ cells, exonucleases and/or helicases other than RecBCD may be able to provide some recombinogenic substrates. Of course, double-strand breaks occurring spontaneously could also initiate a similar pathway (HARRIS *et al.* 1994).

Although we favor the hypothesis that DNA synthesis associated with recombination is producing the mutations, our results do not eliminate the alternative, that recombination is required to preserve mutations occurring by other pathways. There is no such doubt about a recent report that double-strand breaks produced by induction of the HO endonuclease in *S. cerevisiae* are associated with a 100-fold increase in the mutation rate of an adjacent gene (STRATHERN *et al.* 1995). These authors proposed somewhat different models than presented here, one of which also implicated eva-

sion of mismatch repair during Holliday junction resolution.

The production of Lac^+ mutations in nondividing FC40 cells during lactose selection has revealed an unexpected mutagenic mechanism. However, perhaps of more interest to evolutionist is why these mutations appear to be adaptive, *i.e.*, why do they not occur when cells are starving in the absence of lactose (CAIRNS and FOSTER 1991). One possibility is that the low level of lactose metabolism achieved by the unreverted *lacI33-lacZ* allele provides enough energy for nicking at *oriT* and episomal DNA synthesis, but not enough energy to allow the cells to divide (FOSTER and TRIMARCHI 1995; GALITSKI and ROTH 1995; RADICELLA *et al.* 1995). A more intriguing possibility is that, instead or in addition, the recombination-dependent mutagenic process produces a transcribable intermediate that allows wild-type β-galactosidase protein to be produced, and that this then provides the energy to complete the recombinational process. In the absence of a useful error, or in the absence of lactose, the error-containing DNA would be lost. Transcribable intermediates have previously been detected in a closely related phenomenon, *oriT*-dependent recombination between F and the chromosome (YANCEY and PORTER 1985). In the general model presented here, an error made on the transcribed strand, when translocated into the track of doubly unmethylated DNA, could provide such an intermediate.

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LITERATURE CITED

ARRAJ, J. A., T.-H. WU and M. G. MARINUS, 1990 Expression of a DNA methylation (*dam*) gene in *Escherichia coli* K-12. Curr. Microbiol. **20:** 133–136.

ASAI, T., and T. KOGOMA, 1994 D-loops and R-loops: alternative mechanisms for the initiation of chromosome replication in *Escherichia coli*. J. Bacteriol. **176:** 1807–1812.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. G. SEIDMAN *et al.*, 1991 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

CAIRNS, J., and P. L. FOSTER, 1991 Adaptive reversion of a frameshift mutation in *Escherichia coli*. Genetics **128:** 695–701.

CAIRNS, J., J. OVERBAUGH and S. MILLER, 1988 The origin of mutants. Nature **335:** 142–145.

CALOS, M. P., and J. H. MILLER, 1981 Genetic and sequence analysis of frameshift mutations induced by ICR-191. J. Mol. Biol. **153:** 39–66.

CONNOLLY, B., C. A. PARSONS, F. E. BENSON, H. J. DUNDERDALE, G. J. SHARPLES *et al.*, 1991 Resolution of Holliday junctions *in vitro* requires the *Escherichia coli ruvC* gene product. Proc. Natl. Acad. Sci. USA **88:** 6063–6067.

COULONDRE, C., and J. H. MILLER, 1977 Genetic studies of the *lac* repressor. IV. Mutagenic specificity in the *lacI* gene of *Escherichia coli*. J. Mol. Biol. **117:** 577–606.

CSONKA, L. N., and A. J. CLARK, 1979 Deletions generated by the transposon Tn*10* in the *srl recA* region of the *Escherichia coli* K-12 chromosome. Genetics **93:** 321–343.

CUPPLES, C. G., M. CABRERA, C. CRUZ and J. H. MILLER, 1990 A set of *lacZ* mutations in *Escherichia coli* that allow rapid detection of specific frameshift mutations. Genetics **125:** 275–280.

DAMAGNEZ, V., M-P. DOUTRIAUX and M. RADMAN, 1989 Saturation of mismatch repair in the *mutD5* mutator strain of *Escherichia coli*. J. Bacteriol. **171:** 4494–4497.

FOSTER, P. L., 1993 Adaptive mutation: the uses of adversity. Annu. Rev. Microbiol. **47:** 467–504.

FOSTER, P. L., 1994 Population dynamics of a Lac^- strain of *Escherichia coli* during selection for lactose utilization. Genetics **138:** 253–261.

FOSTER, P. L., and J. CAIRNS, 1992 Mechanisms of directed mutation. Genetics **131:** 783–789.

FOSTER, P. L., and J. M. TRIMARCHI, 1994 Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. Science **265:** 407–409.

FOSTER, P. L., and J. M. TRIMARCHI, 1995 Adaptive reversion of an episomal frameshift mutation in *Escherichia coli* requires conjugal functions but not actual conjugation. Proc. Natl. Acad. Sci. USA **92:** 5487–5490.

FOSTER, P. L., G. GUDMUNDSSON, J. M. TRIMARCHI, H. CAI and M. F. GOODMAN, 1995 Proofreading-defective DNA polymerase II increases adaptive mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA. **92:** 7951–7955.

FRIEDBERG, E. C., G. C. WALKER, and W. SIEDE, 1995 *DNA Repair and Mutagenesis*. American Society for Microbiology, Washington, DC.

FROST, L. S., K. IPPEN-IHLER and R. A. SKURRAY, 1994 Analysis of the sequence and gene products of the transfer region of the F sex factor. Microbiol. Rev. **58:** 162–210.

GALITSKI, T., and J. R. ROTH, 1995 Evidence that F plasmid transfer replication underlies apparent adaptive mutation. Science **268:** 421–423.

HARRIS, R. S., S. LONGERICH and S. M. ROSENBERG, 1994 Recombination in adaptive mutation. Science **264:** 258–260.

IWASAKI, H., M. TAKAHAGI, T. SHIBA, A. NAKATA and H. SHINAGAWA, 1991 *Escherichia coli* RuvC protein is an endonuclease that resolves the Holliday structure. EMBO J. **10:** 4381–4389.

KADNER, R. J., and D. M. SHATTUCK-EIDENS, 1983 Genetic control of the hexose phosphate transport system of *E. coli:* mapping of deletion and insertion mutations in the *uhp* region. J. Bacteriol. **155:** 1052–1061.

KALMAN, M., H. MURPHY and M. CASHEL, 1992 The nucleotide sequence of *recG*, the distal *spo* operon gene in *Escherichia coli* K12. Gene **110:** 95–99.

KATHIR, P., and K. IPPEN-IHLER, 1991 Construction and characterization of derivatives carrying insertion mutations in F plasmid transfer region gene, *trbA*, *artA*, *traQ*, and *trbB*. Plasmid **26:** 40–54.

KATO, T., and Y. SHINOURA, 1977 Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156:** 121–131.

KOWALCZYKOWSKI, S. D., D. A. DIXON, A. K. EGGLESTON, S. C. LAUDER and W. M. REHRAUER, 1994 Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:** 401–465.

KUZMINOV, A., 1995 Collapse and repair of replication forks in *Escherichia coli*. Mol. Microbiol. **16:** 373–384.

KUZMINOV, A., E. SCHABTACK, and F. W. STAHL, 1994 χ sites in combination with RecA protein increase the survival of linear DNA in *Escherichia coli* by inactivating Exo V activity of RecBCD nuclease. EMBO J. **13:** 2764–2776.

LEA, D. E., and C. A. COULSON, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

LLOYD, R. G., 1991 Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia coli* K-12 depends on *recG*. J. Bacteriol. **173:** 5414–5418.

LLOYD, R. G., and C. BUCKMAN, 1991 Genetic analysis of the *recG* locus of *Escherichia coli* K12 and of its role in recombination and DNA repair. J. Bacteriol. **173:** 1004–1011.

MANDAL, T. N., A. A. MAHDI, G. J. SHARPLES and R. G. LLOYD, 1993

Resolution of Holliday intermediates in recombination and DNA repair: indirect suppression of *ruvA*, *ruvB*, and *ruvC* mutations. J. Bacteriol. **175:** 4325–4332.

MARINUS, M. G., A. POTEETE and J. A. ARRAJ, 1984 Correlation of DNA adenine methylase activity and spontaneous mutability in *Escherichia coli* K-12. Gene **28:** 123–125.

MILLER, J. H., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

MILLER, J. H., 1985 Mutagenic specificity of ultraviolet light. J. Mol. Biol. **182:** 45–65.

MOUNT, D. W., K. B. LOW and S. J. EDMISTON, 1972 Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112:** 886–893.

MYERS, R. K., and F. W. STAHL, 1994 χ and the RecBCD enzyme of *Escherichia coli.* Annu. Rev. Genet. **28:** 49–70.

OTSUJU, N., H. IYEHARA and Y. HIDESHIMA, 1974 Isolation and characterization of an *Escherichia coli ruv* mutant which forms nonseptate filaments after low doses of ultraviolet light irradiation. J. Bacteriol. **177:** 337–344.

RADICELLA, J. P., P. U. PARK and M. S. FOX, 1995 Adaptive mutation in *Escherichia coli*: a role for conjugation. Science **268:** 418–420.

ROSENBERG, S. M., S. LONGERICH, P. GEE and R. S. HARRIS, 1994 Adaptive mutation by deletions in small mononucleotide repeats. Science **265:** 405–407.

SCHAAPER, R. M., 1989 *Escherichia coli* mutator *mutD5* is defective in the *mutHLS* pathway of DNA mismatch repair. Genetics **121:** 205–212.

SHAH, R., R. J. BENNETT and S. C. WEST, 1994 Genetic recombination in E. coli: RuvC protein cleaves Holliday junctions at resolution hotspots *in vitro.* Cell **79:** 853–864.

SHARPLES, G. J., F. E. BENSON, G. T. ILLING and R. G. LLOYD, 1990 Molecular and functional analysis of the *ruv* region of *Escherichia coli* K-12 reveals three genes involved in DNA repair and recombination. Mol. Gen. Genet. **221:** 219–226.

SHURVINTON, C. E., R. G. LLOYD, F. E. BENSON and P. V. ATTFIELD, 1984 Genetic analysis and molecular cloning of the *Escherichia coli ruv* gene. Mol. Gen. Genet. **194:** 322–329.

SIEGEL, E. C., S. L. WRAIN, S. F. MELTZER, M. L. BINION and J. L. STEINBERG, 1982 Mutator mutations in *Escherichia coli* induced by the insertion of phage Mu and the transposable resistance elements Tn*5* and Tn*10.* Mutat. Res. **93:** 25–33.

SINGER, M., T. A. BAKER, G. SCHNITZLER, S. M. DEISCHEL, M. GOEL *et al.*, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli.* Microbiol. Rev. **53:** 1–24.

SLATER, S., and R. MAURER, 1993 Simple phagemid-based system for generating allele replacements in *Escherichia coli.* J. Bacteriol. **175:** 4260–4262.

STORM, P. K., W. P. M. HOEKSTRA, P. G. DE HAAN and C. VERHOEF, 1971 Genetic recombination in *Escherichia coli.* IV. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Mutat. Res. **13:** 9–17.

STRATHERN, J. N., B. K. SHAFER and C. B. MCGILL, 1995 DNA synthesis errors associated with double-strand-break repair. Genetics **140:** 965–972.

TAKAHAGI, M., H. IWASAKI, A. NAKATA and H. SHINAGAWA, 1991 Molecular analysis of the *Escherichia coli ruvC* gene, which encodes a Holliday junction-specific endonuclease. J. Bacteriol. **173:** 5747–5753.

VINOPAL, R. T., 1987 Selectable phenotypes, pp. 990–1015 in *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology,* edited by F. C. NEIDHARDT, J. L. INGRAHAM, K. B. LOW, B. MAGASANIK, M. SCHAECHTER *et al.*, American Society for Microbiology, Washington, DC.

VOGEL, H. J., and D. M. BONNER, 1956 Acetylornithinase of *E. coli*: partial purification and some properties. J. Biol. Chem. **218:** 97–106.

VOLKERT, M. R., L. J. MARGOSSIAN and A. J. CLARK, 1981 Evidence the *rnmB* is the operator of the *Escherichia coli recA* gene. Proc. Natl. Acad. Sci. USA **78:** 1786–1790.

WEST, S. C., 1994 The processing of recombination intermediates: mechanistic insights from studies of bacterial proteins. Cell **76:** 9–15.

WHITBY, M. C., and R. G. LLOYD, 1995 Branch migration of three-strand recombination intermediates by RecG, a possible pathway for securing exchanges initiated by 3′-tailed duplex DNA. EMBO J. **14:** 3302–3310.

WHITBY, M. C., L. RYDER and R. G. LLOYD, 1993 Reverse branch migration of Holliday junctions by RecG protein: a new mechanism for resolution of intermediates in recombination and DNA repair. Cell **75:** 341–350.

WILKINS, B. M., 1995 Gene transfer by bacterial conjugation: diversity of systems and functional specializations, pp. 59–88 in *Society for General Microbiology Symposium 52: Population Genetics of Bacteria,* edited by S. BAUMBERG, J. P. W. YOUNG, E. M. H. WELLINGTON and J. R. SAUNDERS. Cambridge University Press, Cambridge.

WILLETTS, N., and R. SKURRAY, 1987 Structure and function of the F factor and mechanism of conjugation, pp. 1110–1133 in *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology,* edited by F. C. NEIDHARDT, J. L. INGRAHAM, K. B. LOW, B. MAGASANIK, M. SCHAECHTER *et al.* American Society for Microbiology, Washington, DC.

WILLETTS, N. S., A. J. CLARK and B. LOW, 1969 Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli.* J. Bacteriol. **97:** 244–249.

WU, T.-H., and M. G. MARINUS, 1994 Dominant negative mutator mutations in the *mutS* gene of *Escherichia coli.* J. Bacteriol. **176:** 5393–5400.

YANCEY, S. D., and R. D. PORTER, 1985 General recombination in *Escherichia coli* K-12: *in vivo* role of RecBC enzyme. J. Bacteriol. **162:** 29–34.

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