RecA-independent recombination is efficient but limited by exonucleases

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Genetic recombination in bacteria is facilitated by the RecA strand transfer protein and strongly depends on the homology between interacting sequences. RecA-independent recombination is detectable but occurs at extremely low frequencies and is less responsive to the extent of homology. In this article, we show that RecA-independent recombination in *Escherichia coli* is depressed by the redundant action of single-strand exonucleases. In the absence of multiple single-strand exonucleases, the efficiency of RecA-independent recombination events, involving either gene conversion or crossing-over, is markedly increased to levels rivaling RecA-dependent events. This finding suggests that RecA-independent recombination is not intrinsically inefficient but is limited by single-strand DNA substrate availability. Crossing-over is inhibited by exonucleases ExoI, ExoVII, ExoX, and RecJ, whereas only ExoI and RecJ abort gene-conversion events. In ExoI$^-$ RecJ$^-$ strains, gene conversion can be accomplished by transformation of short single-strand DNA oligonucleotides and is more efficient when the oligonucleotide is complementary to the lagging-strand replication template. We propose that *E. coli* encodes an unknown mechanism for RecA-independent recombination (independent of prophage recombination systems) that is targeted to replication forks. The potential of RecA-independent recombination to mediate exchange at short homologies suggests that it may contribute significantly to genomic change in bacteria, especially in species with reduced cellular exonuclease activity or those that encode DNA protection factors.

DNA rearrangements | DNA repair | genetic recombination | genetic exchange | replication fork repair

The *recA* gene was the first gene discovered to mediate homologous recombination (1) and is highly conserved in bacteria (2). Archaea and eukaryotes also encode structurally and functionally related proteins, RadA and Rad51, respectively, that are similarly required for genetic recombination and DNA repair (3–6). RecA protein forms a helical filament on single-strand DNA (ssDNA) and catalyzes strand pairing and transfer between homologous DNA molecules *in vitro*, processes that initiate homologous recombination (reviewed in ref. 7). In *Escherichia coli*, where its function has been best studied, mutants in *recA* show severe reductions in recombination measured by different types of genetic crosses (reviewed in ref. 8).

ssDNA initiates recombination, and its coating by RecA also likely protects it from nucleolytic attack. *E. coli* possesses four potent exonucleases (ExoI, ExoVII, ExoX, and RecJ) that digest ssDNA. Three of these exonucleases (RecJ, ExoI, and ExoVII) are processive enzymes, and all four exonucleases can degrade DNA at the approximate rate of thousands of bases per min (9–15). ExoI and ExoX degrade DNA strictly in the 3′ to 5′ direction, RecJ digests 5′ to 3′, and ExoVII can degrade a strand of either polarity.

Our previous work suggested that the ssDNA exonucleases (ssExos) of *E. coli* redundantly abort a number of mutational events involving strand annealing. Strand mispairing events, giving rise to mutations and genetic rearrangements, are dramatically elevated in various multiple mutant combinations of the ssExos. (Genes encoding ExoI, ExoVII, ExoX, and RecJ proteins are *xonA*, *xseA*, *exoX*, and *recJ*, respectively.) Events stimulated by ssExo deficiency include frameshift mutations at runs of repeated sequences (16), a template-switch hotspot mutation at an imperfect inverted repeat sequence (17, 18), and RecA-independent rearrangements between larger (100- to 800-bp) tandemly repeated sequences (19, 20).

In this article, we investigate the impact of ssExos on homologous recombination involving cross-overs or gene-conversion-like events with variable amounts of sequence homology. We find that RecA-independent recombination is greatly stimulated in the absence of ssExos, surprisingly to levels comparable to RecA-dependent events. These data suggest that RecA-independent recombination, even at short sequence homology, can be quite efficient in bacteria and may contribute significantly to genomic change.

Results

Features of the Crossing-Over Assay. In previous work, we developed an assay that scores crossing-over between two compatible plasmids sharing homology between 25 and 411 bp in length (21). Cross-over products can be selected by acquisition of tetracycline (Tc) resistance conferred by the recombinant *tetA* gene. We showed that recombination in this assay strongly depended on the extent of shared homology, with rates reaching a plateau of 10^{-5} per cell generation at ≈150 bp. Recombination involving homologies 50 bp or greater depended on the RecA strand transfer protein. The various genetic influences on RecA-dependent crossing-over led us to conclude that the cross-overs detected occur primarily by recombinational repair of ssDNA gaps (see Fig. 1*A*). In that study, RecA-independent recombination was detectable at rates 10^{-8} to 10^{-7}, was influenced only weakly by the extent of homology, and was the sole mechanism contributing to crossing-over at the lowest homology, 25 bp (21). RecA-independent recombination detected in this assay did not depend on recombination factors RecB, RecFOR, RecG, or RuvABC. The nature of RecA-independent cross-over products were consistent with reciprocal exchange: products were heterodimers with one restored *tetA*$^+$ locus, demanded by the selection, and one doubly deleted *tetA* locus, not demanded by selection but expected if exchange was reciprocal (21).

RecA-Independent Cross-Overs Are Sensitive to ssExos in a Redundant Fashion. For mutants of the four known ssExos, we measured crossing-over by using the two-plasmid assay involving the highest and lowest extents of homology, 411 and 25 bp, respectively (Table 1). A mutant in all four exonucleases (ExoI, ExoVII, ExoX, and RecJ) exhibited a pronounced 20-fold ele-

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Abbreviations: ssExo, ssDNA exonuclease; Tc, tetracycline; Ap, ampicillin.

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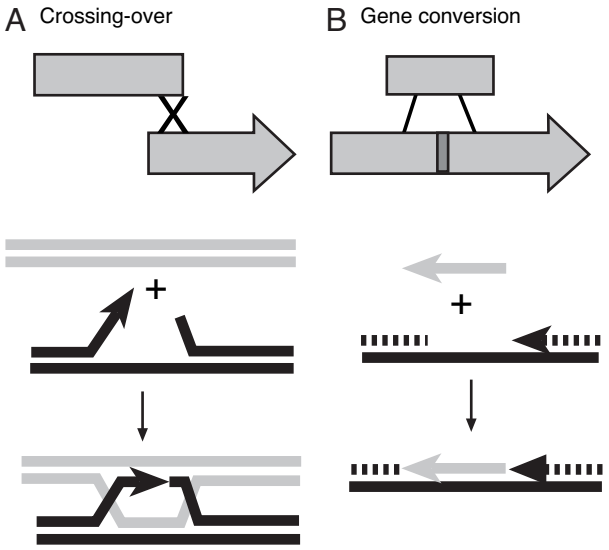

Fig. 1. Diagram of recombination assays and proposed mechanisms. (*A*) Crossing-over assay requires exchange between variable regions of homology. Displacement of single strands from a gap may initiate these events. Such displaced strands are vulnerable to digestion by ExoI, ExoVII, ExoX, or RecJ. (*B*) Gene-conversion assay requires exchange of a patch of genetic information. This mechanism may involve annealing of a single-strand oligonucleotide to the complementary single-strand region in a replication fork. Recombination donor oligonucleotides may be subject to destruction by 5′ exonuclease RecJ or 3′ exonuclease ExoI.

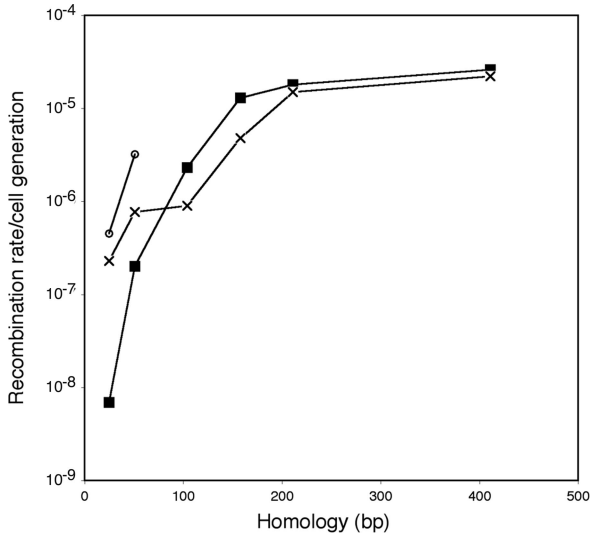

Fig. 2. Cross-over recombination with increasing homology. Recombination rates for wild type (■), ssExo$^-$ (ExoI$^-$ ExoV$^-$ ExoX$^-$ RecJ$^-$) strain (×), or ssExo$^-$ RecA$^-$ strain (○) as a function of homology between the two recombining plasmids.

vation of cross-overs between 25 bp of homology relative to a wild-type strain. A *recA* mutant derivative of this strain, if anything, had even a higher rate, 65-fold over wild type, indicating that these elevated cross-overs occur independently of RecA strand transfer activity. None of the triple, double, or single exonuclease mutants had this strong effect (Table 1 and data not shown) suggesting that any of the four ssExos can efficiently abort crossing-over at 25 bp of homology. We determined cross-over rates for the wild type and the quadruple exonuclease mutant (denoted ssExo$^-$) at multiple extents of homology by using the two-plasmid assay (Fig. 2). The ssExo$^-$ mutant was elevated for cross-over only at the two lowest lengths of homology, 25 and 51 bp, those that show a substantial contribution from the RecA-independent pathway. The rate of cross-over in the ssExo$^-$ RecA$^-$ mutant at 51 bp of homology was higher than the ssExo$^-$ RecA$^+$ strain, indicating that RecA may interfere or compete with RecA-independent exchange. We conclude that the ssExos redundantly inhibit RecA-independent recombination leading to cross-overs. Even at only 51 bp of homology and lacking the RecA gene, the rate of such events ($\approx 3 \times 10^{-6}$) is only modestly lower than RecA-mediated cross-overs at much larger homologies ($\approx 2 \times 10^{-5}$).

Features of the Gene-Conversion Assay. In the previous assay, only recombination leading to cross-overs between the two plasmids could be detected. We designed a comparable two-plasmid assay that detects gene-conversion-like nonreciprocal recombination events, in which a patch of homologous sequence is transferred to the recipient locus (Fig. 1*B*). One plasmid derived from pBR322, pSTL361, carries a 4-bp internal deletion in the *tetA* gene encoding Tc resistance and serves as the recombination recipient. Other compatible plasmids, pSTL362, pSTL363, and STL364, harbor internal pieces of *tetA* that span the deletion on both sides equally, including a total of 80, 200, or 400 nt of homology. A gene conversion from the donor plasmids to the recipient pSTL361 plasmid can remove the deletion and restore *tetA* function. No Tc-resistant isolates ever were obtained from strains carrying only one of the two plasmids, confirming *tetA*$^+$ gene function results from recombination between homologies present on the two coresident plasmids.

ExoI and RecJ Strongly Inhibit RecA-Independent Gene Conversion. Using this two-plasmid assay for gene conversion, we measured recombination rates to Tc resistance in wild-type strains and those lacking various ssExos (Table 2 and Fig. 3). At 80 bp of homology, gene conversion at 10^{-10} per cell generation was

Table 1. Low-homology cross-over recombination in exonuclease-deficient derivatives

Strain	Genotype	25-bp homology*	411-bp homology*
AB1157	Wild type	0.69 (0.36–0.83)	2,600 (330–15,000)
STL6234	*recJ xonA*	1.2 (0.80–2.1)	2,200 (1,100–5,900)
STL6103	*recJ xonA xseA*	1.7 (1.1–5.3)	ND
STL6127	*xonA xseA exoX*	0.88 (0.65–3.5)	ND
STL6140	*recJ xseA exoX*	1.6 (0.14–7.8)	ND
STL6255	*recJ xonA exoX*	1.2 (0.65–3.5)	ND
STL6283	*recJ xonA xseA exoX*	14 (4.9–49)	2,300 (1,100–4,400)
STL6525	*recJ xonA xseA exoX recA*	45 (25–89)	ND

ND, not determined.
*Recombination rate $\times 10^{-8}$ (95% confidence interval).

GENETICS

Table 2. Low-homology gene conversion in exonuclease-deficient derivatives

Strain	Genotype	80-bp homology*
AB1157	Wild type	1.1
STL6064	*xonA*	0.16
STL6001	*xseA*	0.91
STL5908	*recJ*	0.18
STL3817	*recA*::*cat*	0.14
STL6229	*exoX*	0.19
STL6234	*recJ xonA*	450 (190–1,300)
STL6230	*xonA exoX*	<2.7
STL6108	*xonA xseA*	<2.3
STL6233	*recJ exoX*	3.2
STL5998	*recJ xseA*	0.50
STL6125	*exoX xseA*	0.46
STL6518	*recJ xonA recA*::*cat*	820 (400–1,400)
STL6255	*recJ xonA exoX*	700 (220–2,100)
STL6104	*recJ xonA xseA*	490 (120–6,200)
STL6127	*xonA xseA exoX*	3.2
STL6140	*recJ xseA exoX*	<3.7
STL6283	*recJ xonA xseA exoX*	740 (260–1,900)

*Recombination rate $\times 10^{-10}$ (95% confidence interval).

barely detectable in wild-type strains and undetectable in many others. Any strain lacking both ExoI and RecJ exhibited dramatically elevated gene-conversion rates, from 400- to 800-fold. As was seen before for crossing-over at limited homologies, this elevated gene conversion was independent of RecA and even somewhat higher in a *recA* derivative. Sequence analysis of a number of these products confirmed that the deletion mutation marked by NotI in the parental plasmid had been lost and replaced with wild-type *tetA* sequences (our unpublished data).

We compared gene-conversion rates at three lengths of homology: 80 bp, 200 bp, and 400 bp (Fig. 3). For a wild-type strain, increasing homology from 80 to 200 or 400 bp elevated gene-conversion rates over 2 or 3 orders of magnitude, respectively. Gene conversion at the higher homologies showed a strong dependence on RecA, being reduced by 3 orders of magnitude in a *recA* strain. Gene conversion in a *xonA recJ* derivative was elevated relative to wild-type strains at all homologies. For gene conversion at 80 and 200 bp of homology, this recombination was entirely RecA-independent; at 400 bp of homology, gene conversion in ExoI$^-$ RecJ$^-$ strains was reduced ≈10-fold by an additional mutation in RecA. Therefore in a ExoI$^-$ RecJ$^-$ background, RecA-independent gene conversion occurs at comparable efficiencies to events catalyzed by RecA. In the ExoI$^-$ RecJ$^-$ RecA$^-$ background, gene conversion showed no effect of homology: events at 80 bp of homology were as frequent as those at 400 bp. In a ssExo$^+$ background, however, RecA-independent recombination did increase with homology, possibly because of a greater probability of having a segment of the longer homologous sequences escaping exonuclease destruction.

Gene Conversion by Electroporated Oligonucleotides. The stimulation of gene conversion by loss of exonucleases ExoI and RecJ suggested the possibility of introducing one of the recombination partners as a short oligonucleotide by electroporation. In these experiments, various strains harboring the pSTL361 plasmid carrying the *tetA* short internal deletion were electroporated with oligonucleotides that span the deletion, with a total of 80 bases of homology. We transformed two complementary oligonucleotides as individual single strands or annealed as a duplex. To control for efficiency of DNA uptake, we compared the efficiency of acquisition of Tc resistance by the gene-converting oligonucleotide to that of a supercoiled plasmid conferring Tc resistance in parallel transformations (Fig. 4). Control mock-transformations with no added DNA never yielded Tc-resistant progeny, confirming that Tc resistance is gained by recombination with the oligonucleotide.

Gene conversion by oligonucleotides was very inefficient or not detectable in wild-type strains. As we had seen for gene conversion between resident plasmids, gene conversion by electroporated oligonucleotides was strongly enhanced, at least 2 orders of magnitude by concomitant loss of ExoI and RecJ. Gene conversion by oligonucleotide transformation was very modestly augmented by additional inactivation of ExoVII and ExoX, largely independent of RecA (Fig. 4), and not affected in any single ssExo mutant (data not shown.) The oligonucleotide with

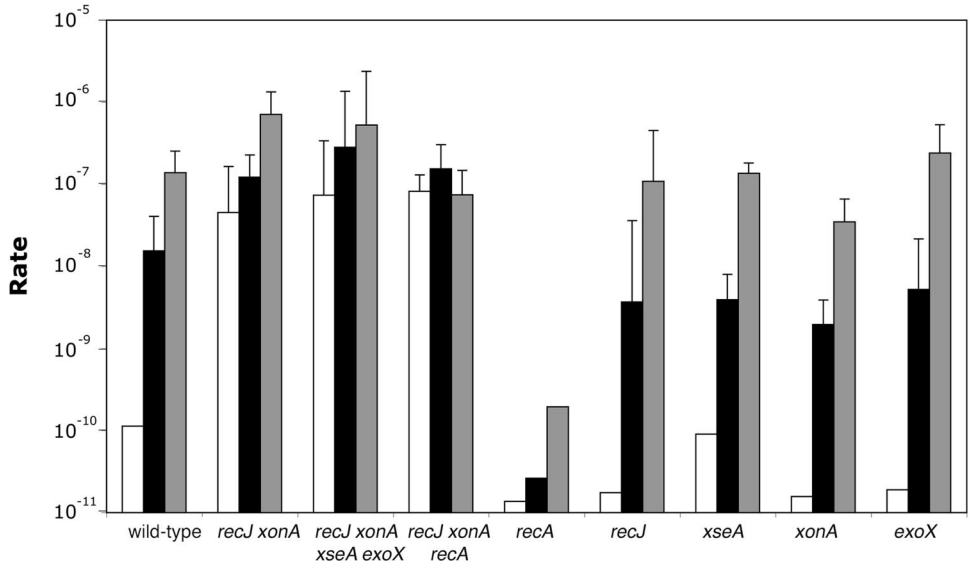

Fig. 3. Gene-conversion recombination in exonuclease-deficient derivatives. Recombination rates were determined with the two-plasmid assay involving three different lengths of homology to *tetA*: 80 bp (white), 200 bp (black), and 400 bp (gray). Gene-conversion rates were calculated by the method of the median (34) for 10–20 independent cultures, with error bars representing 95% confidence intervals (35). Values without error bars were calculated by using the maximum-likelihood method (34) from which confidence intervals cannot be determined.

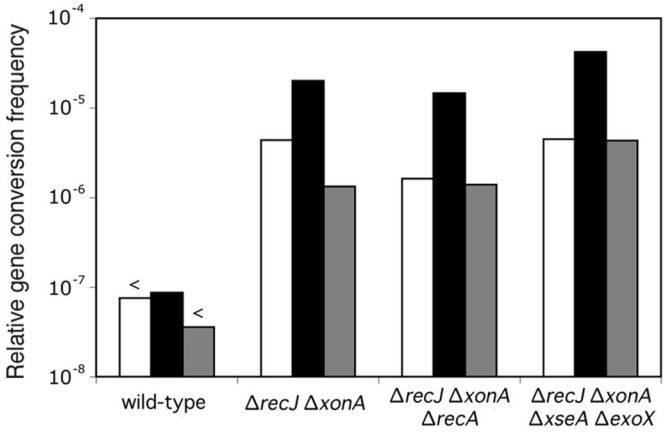

Fig. 4. Relative gene-conversion efficiency by electroporated oligonucleotides. Competent cells of the strain of the indicated genotype harboring pSTL361 (encoding a *tetA* gene with a short internal deletion) were electroporated with single-stranded oligonucleotides that were complementary to the leading strand (white) or lagging strand (black) of the plasmid, or with double-stranded DNA made by annealing the two single-stranded oligonucleotides (gray). Frequencies of acquisition of Tc resistance are relative to the transformation efficiency of the competent cells with normal pBR322. Each value is the median relative frequency of five independent transformations. Bars labeled with < above them indicate maximal values for frequencies for determinations in which no colonies were obtained.

complementarity to the lagging-strand template consistently gave higher gene-conversion frequencies, 5- to 10-fold, relative to the oligonucleotide complementary to the leading-strand template and relative to the annealed double strand.

Discussion

Most reviews of genetic recombination include the statement that the RecA protein is required for all pathways of homologous recombination in bacteria. Our previous work showed that intermolecular homologous recombination via a RecA-independent mechanism could be detected at low rates in the population, raising the possibility that RecA-independent mechanisms could contribute to recombination (21). This report shows that RecA-independent recombination, via gene conversion or crossing-over, is almost as efficient as that catalyzed by RecA, as long as ssExos are absent from the cell. This finding is consistent with the observation of robust *RAD51*-independent mitotic recombination pathways in yeast, where the *RAD52*-encoded protein presumably provides DNA end protection (22).

What is the nature of RecA-independent recombination? Its limitation by ssExos suggests that it employs tails or pieces of ssDNA. Like RecA-dependent recombination, its efficiency increases with homology shared by the interacting molecules, but this dependence is more modest. RecA-dependent recombination shows a strong threshold homology length, at ≈150 bp for crossing-over and between 80 and 200 bp for gene conversion, below which recombination rates fall. RecA-dependent crossing-over may tolerate a shorter homology than gene conversion, because cross-overs at 100 bp of homology were closer to the plateau rate than gene conversion at 80 bp of homology was. RecA-independent recombination, on the other hand, has a lower threshold of ≈50 bp for maximally efficient crossing-over (21) and either no threshold or <80 bp for gene conversion (Fig. 3), which may mean that the base pairing involved in RecA-independent events is more limited, in the range of 50 bp. Our previous work (21) showed that RecA-independent crossing-over resembles RecA-dependent events in several respects: they could be reciprocal in nature, implying a break–rejoin mechanism, and can be inferred to involve Holliday junction intermediates, because both RecA-dependent and -independent cross-overs were stimulated by a mutation in RuvC, a Holliday junction endonuclease. (We explained this latter effect by proposing that RuvC biases resolution to non-cross-over products.)

We have demonstrated efficient RecA-independent sister-chromosome recombination involving a replication template switch that depends on the DnaK chaperone (23). These events are efficient but restricted to sister chromosomes (24) and therefore are intramolecular in nature. We have no evidence that the RecA-independent recombination events demonstrated in this report occur by that mechanism. In assays measuring intermolecular crossing-over at short homologies, *dnaK* mutants are hyperrecombinational rather than hyporecombinational (S.T.L., unpublished data), although effects in the ssExo-deficient genetic backgrounds remains to be tested.

The ssExos appear to play a critical role in scavenging potentially troublesome displaced ssDNA in the cell. This important role in promoting genetic stability has been obscured by the redundant action of the nucleases such that phenotypes are evident only when two or more of the exonucleases are inactivated. We have shown that the ssExos, in various combinations, abort a number of annealing events at short homologies that generate mutations or genetic rearrangements at homopolymeric repeats (16), quasipalindromes (17, 18), or directly repeated tandem DNA sequences (19, 20). *E. coli* has four exonucleases that degrade ssDNA, the most potent of which are RecJ (5′ to 3′) and ExoI (3′ to 5′). These two exonucleases degrade ssDNA at a rate between 1 and 10 kb/min in a processive manner (9–12). ssDNA bound by SSB, a ssDNA binding protein, is not protected and, in fact, is more prone to digestion by ExoI or RecJ (9, 25).

The exonucleases that inhibit RecA-independent genetic exchange differ between cross-over and gene-conversion reactions. We suspect that this is a reflection of differences in the mechanisms of the two types of events. We hypothesize that RecA-independent cross-overs arise as 5′- and 3′-tailed strands are displaced and invade or anneal to a homologous molecule (Fig. 1*A*). Degradation of either of these tails (the 5′ by RecJ or ExoVII and the 3′ by ExoI, ExoVII, or ExoX) can abort the reaction, explaining why elevations are seen only when all four nucleases are inactivated. In contrast, we propose that RecA-independent gene conversions result from released single strands that anneal to single-strand regions in the replication fork (Fig. 1*B*). This mechanism also explains recombination of single-strand oligonucleotides introduced exogenously by electroporation. The preference for inheritance of the oligonucleotide that is complementary to the lagging-strand DNA template (more likely to be single-stranded) suggests an annealing mechanism. Degradation of the oligonucleotide from either the 3′ direction via ExoI or from the 5′ direction via RecJ can destroy these reactants. The lack of effect of ExoVII and ExoX on these substrates may reflect their inefficiency of digestion of these released oligonucleotide substrates, possibly because they are coated by SSB, which does not deter digestion by ExoI or RecJ.

Our results showing gene conversion of electroporated oligonucleotides are reminiscent of reactions stimulated by bacteriophage lambda β protein, which likewise are targeted preferentially to the lagging strand (26, 27). Such oligonucleotide-mediated recombination has been very useful in genetic engineering and, when mediated by β, is not inhibited by the ssExos of *E. coli*, probably because β protein protects the DNA from degradation. Despite this similarity, we have no indication that the RecA-independent recombination observed in our studies is bacteriophage-encoded: AB1157 completely lacks the lambdoid Rac prophage (28) that encodes RecET with similar properties to lambda Exo and β, and we know of no other expressed prophage functions with these properties. Rather, we suggest that *E. coli* possesses intrinsic ability to mediate RecA-independent recombination, as

long as ssDNA can be protected. Whether this recombination requires an annealing–promoting activity, such as that provided by β *in vitro* (29, 30), remains to be determined. We suspect that this pathway could be called into play under certain circumstances to mediate DNA repair simply by inhibition of exonuclease activity. Bacterial genomes are diverse in the known exonuclease orthologs they encode, and DNA end-protection proteins are difficult to predict by sequence alone. We likely underappreciate the role of RecA-independent recombination to genomic change and DNA repair in bacteria: bacterial species may vary considerably in the efficiency of this mechanism, depending on the exonucleases or DNA end-protection proteins they encode.

Materials and Methods

Bacterial Strains and Growth Media. *E. coli* K12 strains used in this study have been described previously (20). All strains are isogenic with AB1157 (31) (which lacks the lambdoid Rac prophage), with deletion mutations in the appropriate exonuclease (20) or a deletion or insertion in the *recA* gene. Bacterial cultures were prepared at 37°C in Luria–Bertani (LB) medium containing 1% (wt/vol) tryptone, 0.5% (wt/vol) yeast extract, 0.5% (wt/vol) NaCl, and 50 μg/ml thymine; plate media contained 1.5% (wt/vol) agar. The following antibiotic concentrations were used for genetic selections: 100 μg/ml ampicillin (Ap), 30 μg/ml chloramphenicol, 60 μg/ml kanamycin, and 7–15 μg/ml Tc.

Plasmids. Plasmids used to measure recombination in this study were described in ref. 21, or their derivations are given below. Plasmids were introduced into appropriate *E. coli* strains by electroporation (32) of purified DNA (miniprep; Qiagen, Valencia, CA). Gene-conversion recombination donor plasmid, pSTL362, carrying 80 bp of *tetA* homology was constructed as follows. The oligonucleotides TetHom1 and TetHom5 (sequences detailed below) were annealed by heating to 100°C and then cooling equal molar amounts of the two oligonucleotides in 40 mM Tris (pH 7.5),10 mM $MgCl_2$, and 50 mM NaCl. The sequence of TetHom1 was 5′-CCACGCCGAA ACAAGCGCTC ATGAGCCCGA AGTGGCGAGC CCGATCTTCC CCATCGGTGA TGTCGGCGAT ATAGGCGCCA GCAA-3′; the sequence of TetHom5 was 5′-GGCCTTGCTG GCGCCTATAT CGCCGACATC ACCGATGGGG AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTTT CGGCGTGGTG CA-3′. The annealed oligonucleotides were ligated with T4 DNA ligase into the pSC101-derived plasmid pWSK129 (33) digested with NotI and PstI. For construction of gene-conversion donor plasmids pSTL363 and pSTL364, with longer lengths of *tetA* homology, the following oligonucleotides were used to amplify 200-bp and 400-bp regions of the *tetA* gene by PCR. Primers for amplification of the 200 bp internal to *tetA* were 5′-GGGGGGAAGC TTTCCTGTGG ATCCTCTACG CCGGACG-3′ and 5′-GGGGGGTCTA GAGAATGGTG CATGCAAGGA GATGGCG-3′; primers amplifying the 400 bp of *tetA* were 5′-GGGGGGAAGC TTGTTCTCGG AGCACTGTCC GACCGCT-3′ and 5′-GGGGGGTCTA GAAGGCTCTC AAGGGCATCG GTCGACG-3′. The PCR products, produced by TripleMaster DNA polymerase (Eppendorf, Westbury, NY) and conditions recommended by the manufacturer, and plasmid pWSK129 each were digested with EcoRV, ligated with T4 DNA ligase, and introduced into XL1-Blue competent cells (Stratagene, La Jolla, CA) via electroporation, selecting for kanamycin resistance. Plasmid DNA was prepared with Qiagen miniprep kits and confirmed by DNA sequence analysis (Molecular Biology Core Facilities, Dana–Farber Cancer Institute, Boston, MA). Gene-conversion recipient plasmid, pSTL361, was produced by PCR-mediated deletion of vector pBR322 by using *Taq*DNA polymerase (Promega, Madison, WI), standard reaction conditions, and oligonucleotides 5′-GGGGGCGGCC GCTCGCCACT TCGGGCTCAT GAGCGCTTG-3′ and 5′-GGGGGCGGCC GCTCTTCCCC ATCGGTGATG TCGGCGATAT AG-3′. The PCR product was digested with NotI, ligated by using T4 DNA ligase, and recovered by electroporation into the strain HMS174, selecting for Ap resistance. The DNA sequence of the entire *tetA* gene in the resulting construct, pSTL361, containing a short deletion marked with a NotI restriction site, was confirmed.

Plasmid × Plasmid Recombination Assays. Crossing-over between two compatible plasmids carrying variable amounts of homology in *tetA* were assayed as previously described for at least 12 independent cultures (21). Recombination rates to Tc resistance per cell generation were calculated by the method of the median (34). Gene-conversion assays were performed similarly with a different set of plasmids. Strains of interest were transformed with recombination recipient plasmid pSTL361 in combination with one of the three recombination donor plasmids: pSTL362, pSTL363, or pSTL364 (having 80, 200, and 400 bp of homology, respectively, to pSTL361). At least 10 independent cultures were grown with aeration in LB plus Ap and kanamycin medium to maintain selection for the plasmids, then plated on LB plus Ap and Tc medium to determine the number of recombinants in the population. Total cells in each culture was determined by serial dilution and plating on LB medium plus Ap. Recombination rates to Tc resistance per cell generation were calculated by the method of the median (34) with 95% confidence intervals as described (35) or, if >40% of the cultures had zero recombinants, by the maximum-likelihood method (34) from which confidence intervals cannot be derived.

Oligonucleotide × Plasmid Recombination Assays. Strains of interest were transformed with recombination recipient plasmid pSTL361, selecting for Ap resistance, and made electrocompetent (32). The cells were transformed with either 1 or 2 ng of the oligonucleotides TetHom1, TetHom5 (see above), or both oligonucleotides annealed by heating to 100°C and slow-cooling. As a control, cells were transformed in parallel with 1 μg or 2 μg of supercoiled pBR322 plasmid DNA purified by using a Qiagen miniprep kit. After transformation, the cells were grown in LB medium for 2 to 2.5 h with aeration and plated on LB plus Ap and Tc medium overnight. Total viable cells in each culture were determined by serial dilution and plating on LB plus Ap medium. For each strain, five independent transformations were performed for each amount of oligonucleotide. Data are expressed as the relative transformation efficiency of the oligonucleotide compared with the plasmid control.

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