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A RecG-Independent Nonconservative Branch Migration Mechanism in *Escherichia coli* Recombination

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To gain insight regarding the mechanisms that extend heteroduplex joints in *Escherichia coli* recombination, we investigated the effect of *recG* and *ruv* genotypes on heteroduplex strand polarity in intramolecular recombination products. We also examined the cumulative effect of mutational inactivation of RecG and single-strand-specific exonucleases on recombination proficiency and the role of Chi sites in RecG-independent recombination. All four strands of the two homologs were incorporated into heteroduplex structures in wild-type cells and in *ruv* mutants. However, in *recG* mutants heteroduplexes were generated almost exclusively by pairing the invasive 3′-ending strand with its complementary strand. To explain the dependence of strand exchange reciprocity on RecG activity, we propose that alternative mechanisms may extend the heteroduplex joints after homologous pairing: a reciprocal RecG-mediated mechanism and a nonreciprocal mechanism, mediated by RecA and single-strand-specific exonucleases. The cumulative effect of *recG* and *recJ* or *xonA* mutations on recombination proficiency and the inhibitory effect of *recJ* and *xonA* activities on heteroduplex formation by the 5′-ending strands are consistent with this proposal.

Biochemical and genetic studies indicate that heteroduplex joint formation in *Escherichia coli* recombination is initiated by pairing of a 3′-ending single-stranded DNA with a double-stranded homolog (5, 9, 29). After pairing, branch migration extends the heteroduplex and, as migration proceeds through a duplex-duplex region, a four-stranded Holliday junction is produced. Resolution of this junction yields products with two complementary heteroduplex structures. One heteroduplex is made by pairing the invasive 3′-ending strand and its complementary strand. The other consists the strands that have been displaced in the primary pairing reaction (Fig. 1A).

Branch migration may be driven by RecA-mediated strand exchange or by the junction-specific helicases RuvAB and RecG (reviewed in references 10, 35, 36, and 39). These two helicases bind three- or four-stranded junctions and catalyze branch migration of RecA-generated recombination intermediates (8, 16, 22, 28, 33, 38). However, RecG and RuvAB differentially affect RecA-catalyzed strand exchange. RecG strongly inhibits RecA-mediated heteroduplex formation, whereas RuvAB has little or no effect. (37). Since RecA-catalyzed strand exchange has a 5′→3′ polarity with respect to the invasive strand (24), the inhibitory effect of RecG suggests an opposite polarity for the RecG-catalyzed reaction. This led to the proposition that RecG-mediated migration of three-stranded junctions secures exchanges that have been initiated at 3′-ending single strands (37).

The subtle effect of *recG* mutations on recombination proficiency suggested the occurrence of a RecG-independent branch migration mechanism. This mechanism may depend on RuvAB, since combinations of *ruv* and *recG* mutations have a synergistic effect (13). However, RecA may also catalyze branch migration in the 3′→5′ polarity, provided that the displaced strands are degraded by single-stranded DNA (ssDNA)-specific exonucleases such as RecJ or exonuclease I (1, 4). This polar strand-exchange mechanism is distinguished from the RecG-catalyzed reaction by the structure of the heteroduplex products. The RecG-catalyzed reaction is conservative, and in a duplex-duplex region it is reciprocal. Conversely, the reaction catalyzed by the coupled activities of RecA and ssDNA-specific exonucleases is nonconservative and nonreciprocal. Thus, the RecG-catalyzed reaction would yield products with two complementary heteroduplex structures, whereas the reaction mediated by RecA and ssDNA-specific exonucleases would yield only one. This heteroduplex would consist of the invasive 3′-ending strand and its homolog (Fig. 1A). Another nonconservative mechanism that may produce only one heteroduplex type is single-strand annealing (SSA) (Fig. 1B). SSA recombination is initiated by resectioning of two double-stranded ends and proceeds by annealing of the complementary strands (11, 12).

If the reciprocity of strand exchange depends on RecG activity, *recG* mutations should inhibit the incorporation of the displaced strands into heteroduplex structures. Here we examine the effect of *recG* genotype on heteroduplex strand polarity in intramolecular recombination products. We also attempt to discriminate between two hypothetical RecG-independent nonconservative mechanisms: SSA and nonreciprocal strand exchange.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* strains used in this study are presented in Table 1. Infected cultures harbored pMB4 (2) and were grown in medium supplemented with ampicillin (100 μg/ml). Infection protocols were as described previously (30). The multiplicity of infection (MOI) is indicated in the figure legends. To inhibit replication of phage DNA that escaped restriction, all strains used in this study were lysogenic to λ(ind⁻).

Chimeric phages. The chimeric phages used in this study are presented in Table 2. All phages harbored intramolecular recombination substrates with a direct terminal repeat, cloned between *Eco*RI sites. Cloned recombination substrates in all phages, except λZS820 (30), had Chi sites at the indicated loci (5). To facilitate separation of the two heteroduplex structures from each other and from the corresponding homoduplexes, an eight-nucleotide *Bgl*II linker was inserted as a heteroallelic marker at the *Xmn*I site on a *luxA* gene (30).

Determination of heteroduplex strand polarity. Heteroduplex strand polarity was determined by polyacrylamide gel electrophoresis and Southern hybridization analysis of total cellular DNA preparations digested by *Pvu*II and *Nde*I (Fig.

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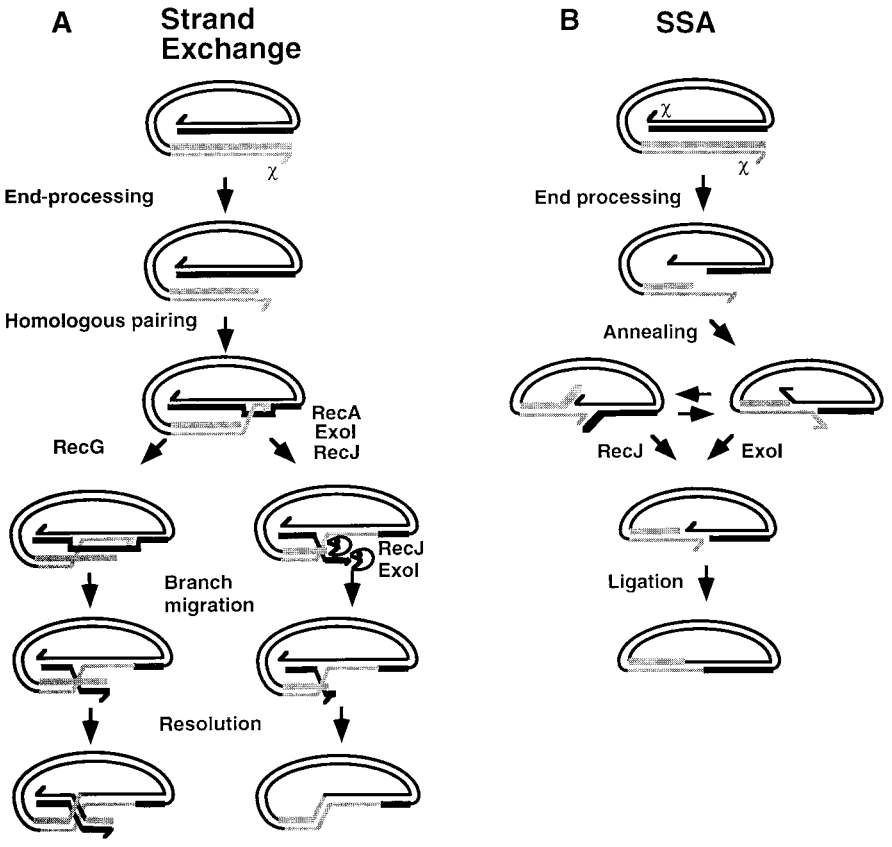


FIG. 1. Hypothetical RecG-dependent and independent branch migration mechanisms. Homologous sequences are designated by black (top) or gray (bottom) parallel lines. 3′-Ending strands of the homologous sequences are narrow and are marked by half arrowheads. 5′-Ending strands are wide. Thus, strands of homoduplex structures have different widths and the same color, and strands of heteroduplex structures have the same width but different colors. (A) After RecA-catalyzed strand invasion, RecG drives the three-stranded junction to a duplex-duplex region, where a Holliday junction is formed. 3′ and 5′ heteroduplexes are then extended by reciprocal strand exchange. In the absence of RecG, polar strand exchange may be catalyzed by RecA, in cooperation with RecJ or exonuclease I (ExoI) that degrades the displaced strands. Both modes of strand exchange depend also on endonucleolytic cleavage of the D-loop. The nonconservative reaction would yield a circular product with a 3′ heteroduplex. (B) A 3′ heteroduplex may be generated by SSA recombination. In this pathway both ends are processed to 3′ overhangs that anneal to a heteroduplex structure.

2A). The location of heteroduplex fragments, consisting of the strands ending 3′ or 5′ at the break, and of the homoduplex fragments are indicated. These locations were determined by using synthetic heteroduplexes as described previously (30).

Physical monitoring of recombination. Total cellular DNA preparations of samples taken at the indicated times after infection were digested by *Sal*I and subjected to Southern blot hybridization as described (30). The kinetics of product formation was determined by phosphorimaging analysis. Radioactivity is presented in arbitrary units.

Determination of recombinant frequency and analysis of plasmid recombination products. To select for Kan^r recombinants, cells were infected by the appropriate chimera phage, and samples taken 60 min after infection were plated on kanamycin-supplemented medium as described earlier (21). To minimize the occurrence of intermolecular recombination events or multiple recombination events within the same infected cell, the MOI was 0.2. The recombinant frequency was defined as the ratio of Kan^r recombinants to infected cells. To determine the percentage of Chi$^+$ products, isolated colonies were inoculated into kanamycin-supplemented liquid medium. Plasmid DNA preparations of overnight cultures were made by the rapid boiling method (7) and were subjected to restriction endonuclease analysis with *Not*I and *Sal*I endonucleases. All substrates had a unique *Sal*I site, and all Chi sites were associated with *Not*I sites (6).

RESULTS

Heteroduplex strand polarity in *recG* mutants. The intramolecular recombination substrate used in this study is a linear DNA fragment with a direct terminal repeat (Fig. 1). The homologous sequences on this substrate are distinguished from each other by an eight-nucleotide insertion in one of them (see Materials and Methods). Hence, their complementary strands can form two chemically distinct heteroduplex structures: one by pairing the strands ending 3′ at the breaks (Fig. 1, narrow lines) and the other by pairing the strands ending 5′ at the breaks (Fig. 1, wide lines). These heteroduplexes are separable by polyacrylamide gel electrophoresis (30) and will be referred to here as the 3′ heteroduplex and 5′ heteroduplex, respectively. Both heteroduplexes are generated by intramolecular recombination, but only the 3′ heteroduplex is incorporated into circular products (5).

The hypothesis depicted in Fig. 1A postulates that RecG is required for reciprocal strand exchange. Consequently, *recG* mutations should lower the ratio of 5′ to 3′ heteroduplexes in recombination products. To examine the effect of *recG* genotype on heteroduplex strand polarity, phage DNA harboring the recombination substrate was delivered into wild-type cells or *recG* mutants by phage infection, and the substrate was released from its carrier by in vivo *Eco*RI restriction (30). The accumulation of 3′ and 5′ heteroduplexes in the infected cells was monitored by Southern hybridization analysis of total cellular DNA preparations from samples taken at various time points after infection (Fig. 2B). As observed previously (5), the 3′ heteroduplex was detected earlier, and its initial rate of accumulation was higher than that of the 5′ heteroduplex. Consistent with the hypothesis in Fig. 1A, a *recG* deletion (Δ*recG263*) markedly lowered the ratio of 5′ to 3′ heteroduplexes. In four of six independent experiments, the 5′ hetero-

TABLE 1. *E. coli* K-12 strains

Strain	Relevant genotype[a]	Reference or source
N2973	*recG162*::Tn*10*	14
N3793	Δ*recG263*::*kan*	18
AM561	Δ*ruvAC65 eda-51*::Tn*10*	19
HRS1200	Δ*ruvC200*::*kan*	25
AC227	λ (ind$^-$)	6
AC258	*recD1009* [λ (ind$^-$)]	6
AC259	*recJ284*::Tn*10* [λ (ind$^-$)]	6
AC261	*recD1011 recJ284*::Tn*10* [λ (ind$^-$)]	5
AC263	*recD1011 xonA2* [λ (ind$^-$)]	5
AC267	*xonA2* [λ (ind$^-$)]	6
AC280	Δ*recG263*::*kan* [λ (ind$^-$)]	P1 N3793 × AC227 to Km^r
AC284	*recD1009* Δ*recG263*::*kan* [λ (ind$^-$)]	P1 N3793 × AC258 to Km^r
AC287	*recD1009 recG162*::Tn*10*[λ (ind$^-$)]	P1 N2973 × AC258 to Tc^r
AC297	*recG162*::Tn*10* [λ (ind$^-$)]	P1 N2973 × AC227 to Tc^r
AC301	Δ*ruvC200*::*kan* [λ (ind$^-$)]	P1 HRS1200 × AC227 to Km^r
AC303	Δ*ruvAC65 eda-51*::Tn*10*[λ (ind$^-$)]	P1 AM561 × AC227 to Tc^r
AC309	*recD1011 recJ284*::Tn*10* Δ*recG263*::*kan* [λ (ind$^-$)]	P1 N3793 × AC261 to Km^r
AC312	*recD1009* Δ*ruvAC65 eda-51*::Tn*10*[λ (ind$^-$)]	P1 AM561 × AC258 to Tc^r
AC314	*recD1011* [λ (ind$^-$)]	6
AC318	*xonA2* Δ*recG263*::kan [λ (ind$^-$)]	P1 N3793 × AC267 to Km^r
AC319	*recJ284*::Tn*10* Δ*recG263*::*kan* [λ (ind$^-$)]	P1 N3793 × AC259 to Km^r
AC320	*recD1011 recJ284*::Tn*10* Δ*ruvC200*::*kan* [λ (ind$^-$)]	P1 HRS1200 × AC261 to Km^r
AC321	*recD1011 xonA2* Δ*recG263*::*kan* [λ (ind$^-$)]	P1 N3793 × AC263 to Km^r
AC322	*recJ284*::Tn10 Δ*ruvC200*::*kan* [λ (ind$^-$)]	P1 HRS1200 × AC259 to Km^r
AC323	*recD1011* Δ*ruvC:200*:*kan*[λ (ind$^-$)]	P1 HRS1200 × AC258 to Km^r
AC324	*recD1011* Δ*recG263*::*kan* [λ (ind$^-$)]	P1 N3793 × AC258 to Km^r
AC325	*recD1011 xonA2* Δ*ruvC200*::*kan* [λ (ind$^-$)]	P1 HRS1200 × AC263 to Km^r
AC326	*xonA2* Δ*ruvC200*::*kan*[λ (ind$^-$)]	P1 HRS1200 × AC267 to Km^r

[a] All strains are isogenic derivatives of AC227. Other markers are *thi-1 his-4* Δ(*gpt-proA*)*62 argE3 thr-1 leuB6 kdgK51 ara14 lacY1 galK2 xyl5 mtl-1 tsx-33 supE44 rpsL31*.

duplex was not detected at any time after infection, while in two it was barely detectable in samples taken 60 min after infection (Fig. 3).

RecG and RuvAB helicases have similar substrate specificities (39), and genetic evidence suggest that the two enzymes act in overlapping pathways that resolve recombination intermediates (13). It was therefore of interest to determine whether mutational inactivation of the RuvABC-mediated strand exchange and resolution pathway would also affect heteroduplex strand polarity. Unlike the Δ*recG* mutation, a Δ*ruvAC* mutation had no detectable effect on heteroduplex strand-polarity (Fig. 2B). The ratio of 5′ to 3′ heteroduplex structures in the Δ*ruvAC* mutant was similar to that in wild-type cells at all time points after infection (Fig. 3A).

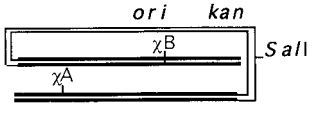
Intramolecular recombination occurs in *recD* mutants at a higher rate than in wild-type cells and is independent of *cis*-acting Chi sites (6). To determine the effect of *recG* and *ruvAC* mutations on heteroduplex strand polarity in *recD* mutants, we infected the appropriate *recD* derivatives by use of λZS820 and monitored the accumulation of 3′ and 5′ heteroduplexes (Fig. 2B). As in *recD*$^+$ cells, a Δ*recG* mutation inhibited the accumulation of recombination products with 5′ heteroduplex structures, whereas the Δ*ruvAC* mutation had no detectable effect (Fig. 3B).

The *recG162* mutation impairs RecG helicase activity but not its ability to bind DNA and hydrolyze ATP. The mutant enzyme cannot catalyze branch migration (27). To examine whether formation of the 5′ heteroduplex depends on RecG helicase activity, we infected *recD1009 recG162* cells with λZS820 and determined heteroduplex strand polarity in recombination products. Like the Δ*recG* mutation, the *recG162* mutation lowered the ratio of 5′ to 3′ heteroduplexes in recombination products. The 5′ heteroduplex was not detectable at 30 min after infection, and at 60 min it represented ca. 10% of the total heteroduplex DNA. This suggests that RecG helicase activity is required for reciprocal strand exchange.

The effect of *xonA*, *recJ*, *recG*, and *ruvAC* mutations on the ratio of 5′ to 3′ heteroduplexes in recombination products is summarized in Fig. 3. Mutational inactivation of RecJ or exonuclease I caused an earlier appearance of the 5′ heteroduplex and an increase in the relative rate of its accumulation (5). Conversely, the 5′ heteroduplex was absent or barely detectable after recombination in *recG* mutants. These data indicate that the invasive 3′-ending strand can be incorporated into heteroduplex products independently of RecG activity but reciprocal strand exchange is RecG dependent. It also suggests that the formation or maintenance of the 5′ heteroduplex is inhibited by RecJ and exonuclease I.

Processing of only one end is sufficient for RecG-independent recombination. To account for the results seen in Fig. 3, we considered two RecG-independent pathways that would not incorporate 5′-ending strands into heteroduplex structures. One pathway involves invasion of double-stranded DNA by a 3′-ending single strand and nonreciprocal strand exchange, catalyzed by RecA and ssDNA exonucleases (Fig. 1A). The other pathway involves pairing by the SSA mechanism (Fig. 1B). Processing of only one end is required for strand invasion, but both ends must be processed to single-stranded overhangs in SSA recombination (11, 12). Chi participates in RecBCD-mediated end processing (10, 32) and is essential for intramolecular recombination by the substrates used in this study (6). Thus, recombination dependence on Chi sites in both homologs would suggest an SSA mechanism. Furthermore, since active Chi sites are invariably lost in end processing, Chi sites on both homologs would be lost in SSA recombination, but only one Chi would be lost in pairing by a strand invasion mechanism. To test these predictions, cells were infected by chimeric phages harboring a linear fragment with a direct terminal repeat and Chi sites on either one or on both homologs. To facilitate isolation of cells that harbor circular recombination products and determination of recombinant frequencies, a pACYC184 replication origin and a *kan*^{r} gene were located between the repeated sequences (31). Consistent with earlier results (6), the *recG* mutation lowered recombinant frequency, and recombination proficiencies of substrates with a single Chi site were lower than that of the substrate with

TABLE 2. Phages harboring recombination substrates[a]



Phage	ori$_{pACYC184}$	Chi at A	Chi at B
λZS820	0	0	0
λRF949	+	0	+
λRF950	+	+	0
λRF951	+	+	+
λRF953	0	+	+

[a] Each Chi site in the schematic representation of the cloned intramolecular recombination substrate consists of three Chi octamers (6). The approximate locations of ori$_{pACYC184}$, the Kan^r gene, and Chi insertion sites (A and B) are indicated.

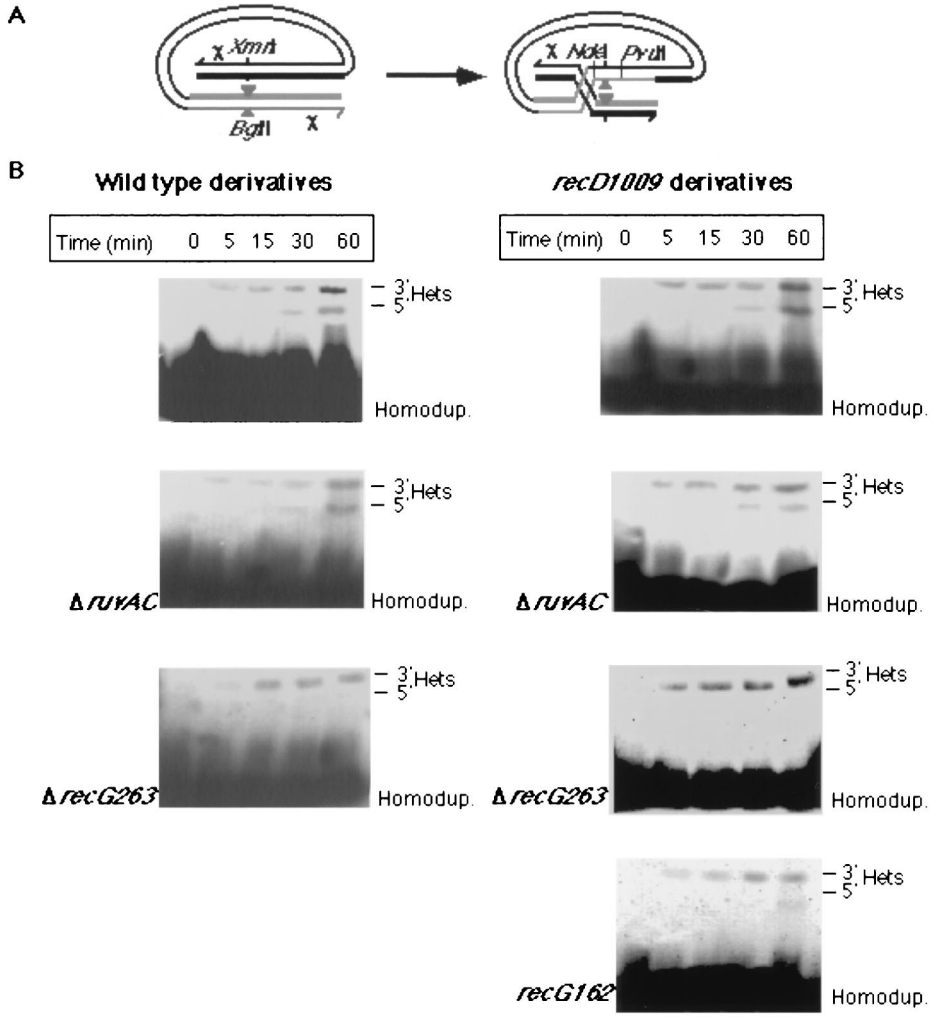


FIG. 2. The effect of *recG* and *ruvAC* mutations on heteroduplex strand polarity. (A) A schematic representation of the phage-delivered intramolecular recombination substrate. The location of the Chi octamers (χ), relevant restriction sites, and the eight-nucleotide *Bgl*II linker (triangles) are indicated. (B) Total cellular DNA preparations (15 μg/lane) of samples taken at the indicated times after infection (MOI = 3) were subjected to Southern hybridization analysis as described in Materials and Methods. Genotypes of the infected cells are indicated. All wild-type derivatives were infected with λRF953, and all *recD1009* derivatives were infected with λZS820. The locations of the electrophoretic bands of the homoduplexes (Homodup.) and the 3′ (3′ Het.) or 5′ (5′ Het.) heteroduplexes are indicated.

two Chi sites. However, the *recG* mutation did not abolish recombination of substrates with a single Chi site. The ratio of recombinant frequency of substrates with a single Chi site to that of substrates with two Chi sites was similar in wild-type cells and *ruvAC* and *recG* mutants (Table 3). We analyzed plasmid recombination products of substrates with two Chi sites for the maintenance of Chi in intramolecular recombination. This was accomplished by restriction analysis of isolated plasmid recombination products with *Sal*I and *Not*I endonucleases. In all substrates employed for this study, *Sal*I had a unique site and *Not*I sites were associated with all Chi sequences (6). The percentage of products that maintained a single Chi site in recombination was not affected by the *recG* or *ruvAC* genotypes (Table 4). These results suggest a *recG*-independent recombination mechanism that involves processing of only one end and are therefore inconsistent with SSA recombination.

A cumulative effect of *recG* and *xonA* or *recJ* mutations on recombination kinetics. If RecJ and exonuclease I participate in a mechanism that functionally overlaps RecG activity, *xonA* or *recJ* mutations should lower recombination proficiency of *recG* mutants. To test this prediction, we compared recombination proficiencies in *xonA recG* or *recJ recG* double mutants to those in *recG*, *recJ*, and *xonA* mutants. *Eco*RI-expressing cells of the appropriate genotypes were infected by λRF953, and the accumulation of recombination products was monitored by Southern hybridization as described before (30). Consistent with earlier results (6), a *recJ* mutation did not affect recombination kinetics, while *xonA* or *recG* mutations had only a partial effect. However, rates of accumulation of recombination products in *recJ recG* or *xonA recG* double mutants were markedly lower than those in any of the single mutants (Fig. 4A). We also examined the combined effect of a *recG* with *xonA* or *recJ* mutations in *recD* mutants (Fig. 4B). RecJ plays a role in RecD-independent recombination, presumably by generating the invasive 3′-ending strand at the presynaptic stage (5, 15, 17). *recG*, *xonA*, and *recJ* mutations lowered the recombination proficiency in *recD* mutants, and a combination of *recG* and *xonA* mutations had a cumulative effect. Significantly, recombination products were not detectable in *recD recG recJ* triple mutants at any time after infection (three independent experiments). We also examined the combined effect of *ruvC*

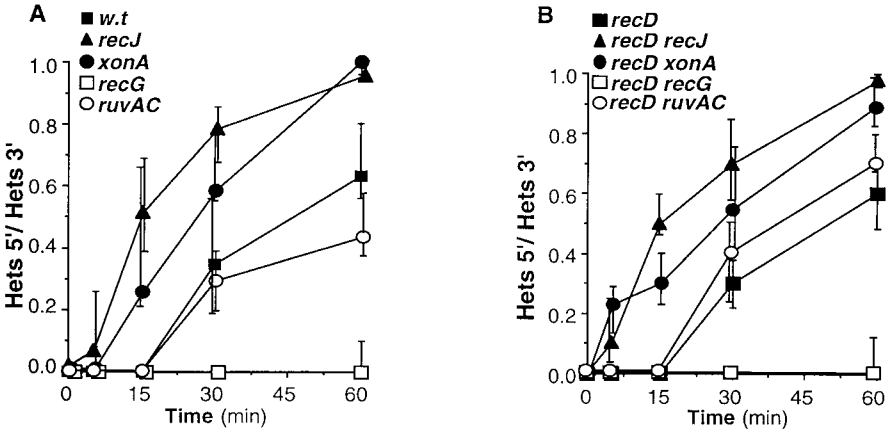


FIG. 3. Effect of *xonA*, *recJ*, Δ*ruvAC*, and Δ*recG* mutations on the ratio of 5′ to 3′ heteroduplexes in intramolecular recombination products. The ratios of 5′ to 3′ heteroduplexes in DNA preparations of samples taken at the indicated times after infection are presented. Radioactivity was determined by phosphorimaging analysis of Southern hybridization patterns like the ones presented in Fig. 2. Relevant genotypes are indicated. Each value is a median of at least three independent experiments. The error bars represent the range.

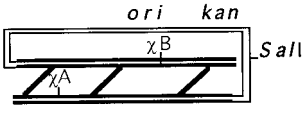
with *recJ* or *xonA* mutations on recombination kinetics. Inactivation of either one of the two exonucleases lowered the recombination proficiency in *ruvC* mutants (Fig. 4C and D).

DISCUSSION

We tested predictions of a hypothesis that postulates alternative branch migration mechanisms in *E. coli* recombination: a conservative mechanism catalyzed by RecG helicase and a nonconservative mechanism catalyzed by RecA and ssDNA-specific exonucleases (Fig. 1A). Consistent with this hypothesis, the *recG* genotype affected heteroduplex strand polarity in intramolecular recombination products. All four strands of the recombining homologs were incorporated into heteroduplex products in *recG*$^+$ cells, whereas in *recG* mutants the 5′-ending strands were absent or barely detectable from heteroduplex structures. Unlike the *recG* mutations, a Δ*ruvAC* mutation did not affect heteroduplex strand polarity. This phenotypic difference between *recG* and *ruv* mutants supports the proposition that RuvAB and RecG are not redundant activities fulfilling the same function in recombination (39). It also suggests that, in vivo, the reciprocity of strand exchange depends on RecG but not on RuvAB. One intriguing possibility is that RecG acts on three-stranded intermediates, generated by RecA-mediated strand invasion, and promotes branch migration into a duplex-duplex region. Subsequently, RuvAB drives the four-stranded Holliday junction to a preferred RuvC cleavage site (37).

What mechanism drives the primary three-stranded junction in the absence of RecG activity? Indirect evidence suggested that the polar strand exchange reactions catalyzed by the coupled activities of RecA and ssDNA-specific exonucleases (1, 4) have a biological relevance. A role for RecJ and exonuclease I in recombination was suggested by the inhibitory effect of *recJ* and *xonA* mutations on "short homology" transduction (20), phage DNA recombination (23), conjugational recombination (34), and intramolecular recombination of linear substrates (6). These exonucleases may act at the presynaptic stage of the recombination pathway by processing the double-stranded DNA ends to single-stranded overhangs or at the postsynaptic stage by conferring a 3′→5′ polarity on RecA strand exchange activity (20, 23). Since synapsis involves pairing of a 3′-ending strand, it is unlikely that exonuclease I acts at the presynaptic stage. Furthermore, a role for RecJ and exonuclease I in a nonconservative branch migration mechanism is suggested by the observation that mutational inactivation of these enzymes increases the ratio of 5′ to 3′ heteroduplexes in intramolecular recombination products (reference 5 and Fig. 3). These findings are consistent with a mechanism that involves cleavage of the anticomplementary strand in the D-loop intermediate by a junction-specific endonuclease (3) and migration of the three-stranded junction by the combined activity of RecA and ssDNA-specific exonucleases. The effect of *recJ* and *xonA* mutations on heteroduplex strand polarity (5) suggests that this mechanism may play a role in *recG*$^+$ cells.

TABLE 3. A Chi site on one homolog is sufficient for intramolecular recombination in *recG* and *ruvAC* mutants[a]



Strain	Genotype	% Frequency (range) of Kan^r recombinants		
		Chi at A and B	Chi at A	Chi at B
AC227	Wild type	10.3 (10–12.1) [1.0]	3.0 (2.4–3.7) [0.3]	1.7 (1.2–2.2) [0.2]
AC297	*recG162*	3.5 (2.5–3.7) [1.0]	0.8 (0.6–0.9) [0.2]	0.4 (0.3–0.4) [0.1]
AC303	Δ*ruvAC*	4.1 (3.7–6.7) [1.0]	1.4 (1.1–1.6) [0.3]	0.6 (0.5–0.9) [0.1]

[a] The approximate locations of Chi sites, ori$_{pACYC184}$ (ori), and the Kan^r gene are indicated. Kan^r recombinants were scored as described in Materials and Methods (MOI = 0.2). The median and range (in parentheses) of at least three independent experiments are presented. For each genotype, values in brackets represent the ratios of recombinant frequencies for the indicated substrate to that for the substrate with two Chi sites.

TABLE 4. A Chi site is incorporated into intramolecular recombination products in *recG* and *ruvAC* mutants[a]

Strain	Genotype	% Chi$^+$ products (range)
AC227	Wild type	36 (35–36)
AC297	*recG162*	44 (42–45)
AC303	Δ*ruvAC*	39 (35–45)

[a] λRF953 (see Table 2) was used in all experiments. The percentage of Chi$^+$ recombination products was determined as described in Materials and Methods and is presented as the median and range of at least three independent experiments.

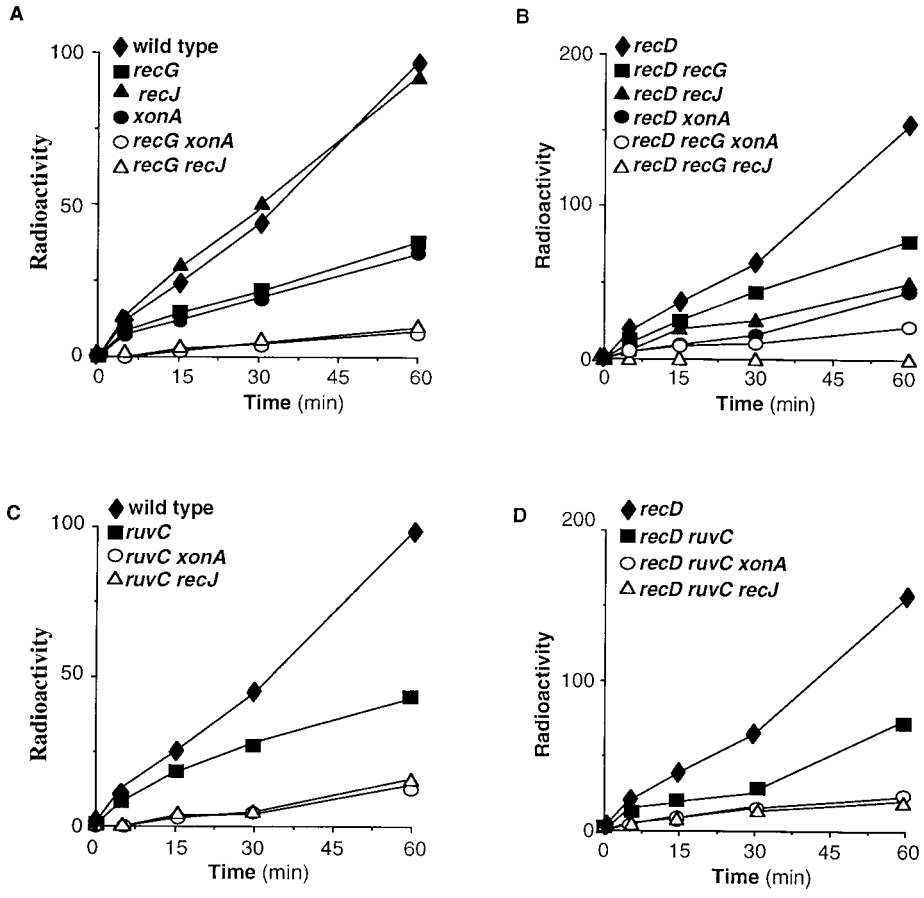


FIG. 4. The effect of *xonA*, *recJ*, *recG*, and *ruvC* mutations, and the indicated double mutations, on intramolecular recombination proficiency. Substrates were released from infecting Chimera phage by in vivo restriction and samples were taken at the indicated times after infection. All wild-type derivatives were infected with λRF953, and all *recD1011* derivatives were infected with λZS820 (MOI = 2). *Sal*I-digested total cellular DNA preparations were subjected to Southern hybridization as described in Material and Methods. The kinetics of product formation were determined by phosphorimaging analysis of Southern hybridization patterns. The radioactivity is presented in arbitrary units.

An alternative interpretation of the data presented in Fig. 2 is that, in the absence of RecG, intramolecular recombination is by the SSA mechanism that incorporates only the 3′-ending strands into a heteroduplex structures. This possibility seems unlikely since processing of only one end is sufficient for intramolecular recombination (Tables 3 and 4).

Mutations in *ruvC*, *recG*, *xonA*, or *recJ* genes have only a partial effect on recombination, whereas mutational inactivation of any pair of these genes causes recombination deficiency (references 5, 13, 20, 23, and 34 and the present study). These data are consistent with the notion of a functional overlap between the activities of the respective gene products in recombination. Thus, RecG or a combination of RecA and ssDNA-specific exonucleases may act on a RecA-generated D-loop to extend the heteroduplex structure. The synergistic effect observed in *recG ruv* double mutants and the cumulative effect of *ruvC* with *recJ* or *xonA* mutations suggest that RuvABC acts in both pathways. RuvABC may act in both pathways by resolving branched recombination intermediates. It is also possible that, in the absence of RecG, RuvAB acts in concert with ssDNA-specific exonucleases to promote unidirectional strand exchange. The partial effect of *ruvABC* mutations on recombination proficiency suggests an alternative mechanism that resolves three- or four-stranded recombination intermediates. Such mechanism may involve a functional analog of the λ Rap protein (26).

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