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Genetics

Heteroduplex joint formation in *Escherichia coli* recombination is initiated by pairing of a 3′-ending strand

(genetic recombination/RecBCD/RecD/strand exchange/RecJ)

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ABSTRACT **The formation of heteroduplex joints in *Escherichia coli* recombination is initiated by invasion of double-stranded DNA by a single-stranded homologue. To determine the polarity of the invasive strand, linear molecules with direct terminal repeats were released by *in vivo* restriction of infecting chimeric phage DNA and heteroduplex products of intramolecular recombination were analyzed. With this substrate, the invasive strand is expected to be incorporated into the circular crossover product and the complementary strand is expected to be incorporated into the reciprocal linear product. Strands of both polarities were incorporated into heteroduplex structures, but only strands ending 3′ at the break were incorporated into circular products. This result indicates that invasion of the 3′-ending strand initiates the heteroduplex joint formation and that the complementary 5′-ending strand is incorporated into heteroduplex structures in the process of reciprocal strand exchange. The polarity of the invasive strand was not affected by *recD, recJ*, or *xonA* mutations. However, *xonA* and *recJ* mutations increased the proportion of heteroduplexes containing 5′-ending strands. This observation suggests that RecJ exonuclease and exonuclease I may enhance recombination by degrading the displaced strands during branch migration and thereby causing strand exchange to be unidirectional.**

The heteroduplex joint is a key intermediate in genetic recombination. Enzymatic studies indicate that formation of the heteroduplex joint in *Escherichia coli* involves polymerization of RecA protein on single-stranded DNA (ssDNA) and pairing of the RecA–ssDNA nucleoprotein complex with a double-stranded DNA homologue (reviewed in refs. 1 and 2). The ssDNA substrate for the homologous pairing reaction is generated at the presynaptic stage of recombination by RecBCD-mediated processing of a double-stranded DNA end. This activity is controlled by a cis-acting sequence (5′-GCTGGTGG-3′) named Chi that interacts with RecBCD in an orientation-dependent manner (reviewed in refs. 1 and 3–5). The primary heteroduplex joint may be extended by branch migration, and if migration proceeds into a duplex–duplex region, a four-stranded Holliday intermediate that contains complementary heteroduplex structures is produced. Resolution of this intermediates generates crossover or noncrossover recombination products (6).

The multiple activities of RecBCD (7–9) and the ability of RecA to catalyze joint formation at the 3′ or 5′ ends of ssDNA (10) allow for several hypothetical mechanisms of recombination (9, 11–13). One distinguishing feature of these mechanisms is the polarity of the invasive strand that initiates heteroduplex joint formation. Although some models postulate pairing solely by the 3′-ending strand (9, 12, 14), others argue that pairing by either the 3′- or the 5′-ending strand would be productive (3, 11).

Several enzymes may influence the polarity of the strand incorporated into the primary heteroduplex joint. RecBCD may generate an intermediate with a 3′-ending strand by strand-specific nicking at Chi and unwinding the 3′-ending strand (15) or by switching the polarity of its DNA degradation activity from 3′→5′ to 5′→3′ (9). Both mechanisms depend on the activity of the RecD subunit and on the interaction of RecBCD with Chi (16, 17). An alternative model postulates that the dissociation or inactivation of RecD at Chi attenuates RecBCD exonuclease activity, and subsequent unwinding of the double-stranded DNA by RecBC(D) helicase produces an intermediate with frayed single-stranded ends (3, 13). Processing of this putative intermediate by RecJ exonuclease or by exonuclease I would generate substrates for the homologous pairing reaction with 3′- or 5′-ending single-stranded overhangs, respectively (18, 19). If ssDNA ends of both polarities are available, RecA may promote joint formation at either end (10). However, ssDNA-binding protein selectively inhibits joint formation at the 5′ end by replacing RecA that dissociates from the RecA–DNA nucleoprotein in a 5′ to 3′ direction (10, 20, 21). RecO and RecR may abate the bias for 3′ pairing by preventing RecA replacement by ssDNA-binding protein and/or by helping reform the RecA filaments (21). In a RecA-mediated exchange reaction between a ssDNA circle and a linear double-stranded DNA, exonuclease I affects end preference of joint molecule formation (22) and RecJ exonuclease enhances exchange of the 3′-ending strand (23). Both exonucleases are postulated to drive unidirectional branch migration by degrading the displaced strands. RecG-mediated strand exchange also may affect strand polarity of heteroduplex joints by securing exchanges initiated by 3′ end invasion and aborting exchanges initiated by 5′ end invasion (24). The biological relevance of these enzymatic activities is not yet understood.

We wished to determine the polarity of the ssDNA that initiates heteroduplex joint formation *in vivo*. To this end, we released linear DNA molecules with direct terminal repeats within *E. coli* cells by *in vivo* restriction of infecting chimeric phage DNA. After intramolecular recombination by the released substrates, we determined strand polarity of heteroduplex structures in unreplicated recombination products. We investigated the effect of some of the enzymatic activities described above on the polarity of strand exchange by analyzing heteroduplex strand polarity in the appropriate mutants. Results indicate that, in *E. coli* recombination, pairing of a 3′-ending strand initiates the formation of heteroduplex joints. The strand of the opposite polarity may be incorporated into

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Abbreviation: ssDNA, single-stranded DNA.
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a heteroduplex structure during branch migration by reciprocal strand exchange.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. *E. coli* strains (Table 1) were grown on LB medium (25). Infected cultures harbored pMB4 (26), and were grown in media supplemented with ampicillin (100 μg/ml). Infection protocol was as described (27).

Chimeric Phage. Chimeric phage with intramolecular recombination substrates, cloned between *Eco*RI sites, were λZS820 (27) or λRF953 (30). Both phages harbor intramolecular recombination substrates with a direct repeat of *vibrio fischeri luxA luxB* genes. An 8-nt *Bgl*II linker was inserted into the *Xmn*I site in one copy of the *luxA* genes as a heteroallelic marker. λRF953 was designed for recombination in wild-type cells. The cloned substrate in this phage has six functional Chi sites, three at each of two loci. These loci were at a distance of 837 and 1,567 nt from the respective *Eco*RI-induced breaks (30). To stabilize heteroduplex structures on recombination products, the cloned substrates were devoid of replication origins.

Physical Monitoring of Recombination. Cells were infected at a moi of 2. Total cellular DNA preparations of samples taken at the indicated times after infection were digested by *Sal*I endonuclease and subjected to Southern blot hybridization as described (27). The kinetics of product formation (Fig. 4) were determined by phosphorimaging analysis. Radioactivity is presented in arbitrary units.

Determination of Heteroduplex Strand Polarity. Heteroduplex strand polarity was determined by PAGE and Southern hybridization analysis of DNA preparations, digested by *Pvu*II and *Nde*I, as described (27). An artificial mixture of homoduplex and heteroduplex fragments with a single mismatch at the *Xmn*I site (eight mispaired bases) was used as a standard. To distinguish between the two complementary heteroduplexes, the separated fragments were hybridized to two complementary 19-mer probes, specific for the mutated sequence. These probes consisted of a 6-nt sequence of the *Bgl*II linker and the adjacent 13-nt sequence on the *luxA* gene (27). To increase the sensitivity of the assay, cells were infected at a moi of 5. Where indicated, total cellular DNA preparations were analyzed. To enrich for nonchromosomal DNA, the clear lysate procedure (31) was used and DNA was purified from the clear lysates by phenol-chloroform extractions and cold ethanol precipitation (32).

Table 1. *E. coli* strains used

Strain	Relevant genotype*	Reference or source
BT125	*recD1011*	29
WA821	*recD1011 xonA2*	29
WA822	*recD1011 xonA2 recJ284::Tn10*	29
WA735	*recD1011 recJ284::Tn10*	29
AC227	*rec*$^+$ [λ(ind$^-$)]	30
AC259	*recJ284::Tn10* [λ(ind$^-$)]	30
AC260	*xonA2 recJ284::Tn10* [λ(ind$^-$)]	30
AC261	*recD1011 recJ284::Tn10* [λ(ind$^-$)]	This work
AC262	*recD1011 xonA2 recJ284::Tn10* [λ(ind$^-$)]	This work
AC263	*recD1011 xonA2* [λ(ind$^-$)]	This work
AC267	*xonA2* [λ(ind$^-$)]	This work
AC275	*recB21 recC22* [λ(ind$^-$)]	30
AC278	*recA13* [λ(ind$^-$)]	30
AC314	*recD1011* [λ(ind$^-$)]	This work

*All strains are isogenic derivatives of AB1157 (28). 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.*

Enzymes. Enzymatic reactions with restriction endonucleases were performed as directed by the suppliers. RecBCD enzyme was a gift from Andrew F. Taylor and Gerald R. Smith (Fred Huchinson Cancer Research Center, Seattle). Digestion of clear lysates and total cellular DNA preparations by RecBCD enzyme was performed with 0.5–1.0 units of enzyme/μg DNA, by the method of Lackey and Linn (33). Reaction was stopped with 20 mM EDTA, and DNA was purified by phenol-chloroform extractions and ethanol precipitation or dialysis (32).

RESULTS

Experimental Design and Terminology. To determine the polarity of the invasive strand in the homologous pairing reaction, we analyzed heteroduplex strand polarity in unreplicated products of intramolecular recombination. The rationale of this approach is illustrated in Fig. 1. The recombination substrate is a linear DNA molecule with a direct terminal repeat. The complementary strands of the repeated sequence (parallel lines) can form two chemically distinct heteroduplex types: one by pairing the strands ending 3′ at the *Eco*RI-induced breaks (narrow lines) and the other by pairing the strands ending 5′ at the breaks (wide lines). These heteroduplex types will be referred to as "3′ heteroduplex" and "5′ heteroduplex," respectively. Both heteroduplex types are expected if strand invasion is not polarity-specific (Fig. 1*A*) or if polarity-specific strand invasion is followed by a reciprocal strand exchange (Fig. 1*B*). However, in the latter case, only the invasive strand will be incorporated into a heteroduplex struc-

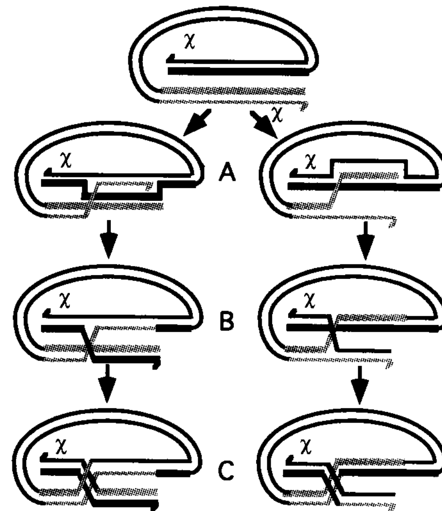


FIG. 1. In intramolecular recombination by a linear substrate with a direct terminal repeat, only the invasive strand is incorporated into heteroduplex structures on the circular product. Homologous sequences are designated by black (top) or gray (bottom) parallel lines. 3′-ending strands of the homologous sequences are narrow, and 5′-ending strands are wide. Thus, strands of homoduplex structures have different widths and the same color, whereas strands of heteroduplex structures have the same width but different colors. The possibility that strand invasion is not polarity-specific is considered, and invasion of upper homolog by the 3′ (left)- or 5′ (right)-ending strands of lower homolog is diagrammed. One heteroduplex type is produced by a single-strand exchange (*A*), but two heteroduplex types are expected if pairing is followed by a reciprocal strand-exchange (*B*). However, after resolution of the Holliday junction (*C*), only the invasive strand will be incorporated into heteroduplex structures on the circular recombination product. The alternative resolution of the Holliday junction is not presented because this resolution would yield a linear product with the two heteroduplex types. Only invasions from the lower ends are illustrated. Invasion from the upper ends gives similar results.

ture in the circular product. The complementary heteroduplex type would be in the reciprocal linear product (Fig. 1*C*). Thus, by analyzing heteroduplex strand polarity in unreplicated products of intramolecular recombination, we should be able to determine the polarity of the invasive strand in the homologous pairing reaction.

The substrate used in this study is a 13.2-kb long linear DNA fragment with a direct terminal repeat of 3.5 kb, consisting of mutated *vibrio fischeri luxA luxB* genes. This substrate was cloned in a phage λEMBL4 vector, delivered by infection into *E. coli* cells, and released by *in vivo Eco*RI restriction of the chimeric phage DNA. To facilitate electrophoretic separation of the two heteroduplex structures from each other and from the corresponding homoduplexes, an 8-nt *Bgl*II linker was inserted as a heteroallelic marker at the *Xmn*I site (Fig. 2*A*) on a *luxA* gene (27). In wild-type cells, intramolecular recombination by this substrate involves a strand exchange mechanism and depends on Chi octamer sequences, a double-strand break, and functional RecA and RecBCD enzymes (30).

Heteroduplex Strand Polarity in Recombination Products. Linear intramolecular recombination substrates (RF953) were released within *E. coli* cells (AC227) by *in vivo* restriction of infecting chimeric phage (λRF953), and samples were taken at time intervals after infection. To enrich for nonchromosomal DNA, clear lysates (31) were prepared and subjected to phenol-chloroform extractions. The purified DNA was digested by *Pvu*II and *Nde*I (see Fig. 2*A*) and analyzed for heteroduplex strand polarity by PAGE and Southern blot hybridization (Fig. 2*B*). Only one heteroduplex band was observed in clear lysate preparations, and its electrophoretic mobility was similar to that of a synthetic 3′ heteroduplex. The complementary 5′ heteroduplex was not detectable in clear lysates of samples taken up to 60 min after infection.

The clear lysate procedure originally was designed to enrich for circular DNA molecules (31), and the recovery of noncircular plasmid DNA by this method is inefficient (34). Therefore, we considered the possibility that the 5′ heteroduplex structures were produced and subsequently lost in the purification procedure. To test for this possibility, we examined heteroduplex strand polarity in total cellular DNA preparations of infected cells (Fig. 2*C*). Both 3′ and 5′ heteroduplex products were observed in total cellular DNA preparations. However, the accumulation of 3′ heteroduplex structures started earlier than 5 min after infection, whereas 5′ heteroduplex structures were not detectable until 30 min after infection. Heteroduplex bands were not observed in *recA* and *recB recC* mutants, thus indicating that these structures are products of recombination.

The preferential loss of the 5′ heteroduplex from the clear lysate preparations suggested that the two heteroduplex types were located on different molecular structures. Evidence for the incorporation of the 5′-ending strand into noncircular recombination products is presented below (see Fig. 6).

Heteroduplex Strand Polarity in *recD* Mutants. Chi-dependent end processing by RecBCD may discriminate against pairing of the 5′-ending strand in a RecD-dependent manner (9, 15–17). It was therefore of interest to test for a possible effect of *recD* mutations on the polarity of the strands incorporated into the heteroduplex structures. A lysogenic *recD1011* (AC314) strain, harboring pMB4 plasmids, was infected by λZS820. Samples were taken at time intervals after infection, and the distribution of the 3′ and 5′ heteroduplexes in clear lysates and in total cellular DNA preparations was determined (Fig. 3). The bias for the 3′ heteroduplex, observed in wild-type cells, also was observed in *recD* mutants. The 5′ heteroduplex was not detectable in clear lysates (Fig. 3*A*). It was also absent from total cellular DNA preparations of samples taken before 30 min after infection (Fig. 3*B*). The ratio of 5′ to 3′ heteroduplex structures increased in time after the

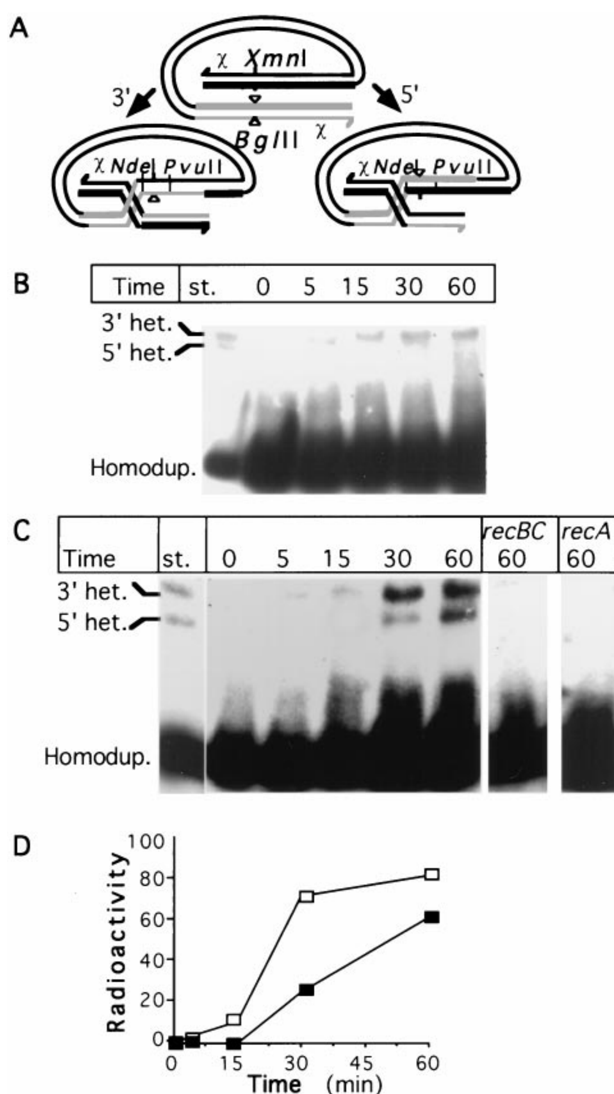


FIG. 2. Heteroduplex production in λRF953-infected AC227[pMB4] cells. Equal amounts (15 μg/lane) of DNA from clear lysates (B) or total DNA preparations (20 μg/lane) (*C*) of samples taken at the indicated time after infection were digested by *Pvu*II and *Nde*I restriction endonucleases and subjected to PAGE and Southern blot hybridization as described in text. Artificial complementary heteroduplexes (st.) were prepared as described (27) and identified as described in *Materials and Methods*. Digested samples from infected *recBC* and *recA* mutants are presented as negative controls. The schematic diagram (*A*) illustrates the linear substrate, released by *in vivo* restriction of the infecting phage. The locations of the Chi octamers (χ), relevant restriction sites, and the *Bgl*II linker (triangles), inserted as a heteroallelic marker at the *Xmn*I site, are indicated. The expected circular and linear products of recombination, initiated by invasion of the 3′ (left)- or 5′ (right)-ending strands of the lower homolog (see Fig. 1) and mismatches on the circular products are indicated. The locations of the electrophoretic bands of the homoduplexes (Homodup.) and the heteroduplexes made by pairing the strands ending 3′ (3′ het.) or 5′ (5′ het.) at the *Eco*RI-induced breaks are indicated. The kinetics of 3′ (□) and 5′ (■) heteroduplex production in total cellular DNA preparations (*D*) was determined by phosphorimaging analysis of the Southern blot presented in *C*.

appearance of the 5′ heteroduplex (see Fig. 5). Heteroduplex bands were not observed in a *recA recD* mutant.

The Effect of Single-Strand-Specific Exonucleases on Heteroduplex Strand Polarity. The synergistic effect of *xonA* and *recJ* mutations on the efficiency of "short homology" transduction (18) and phage DNA recombination (19) suggested a role for exonucleases I and RecJ exonuclease in recombina-

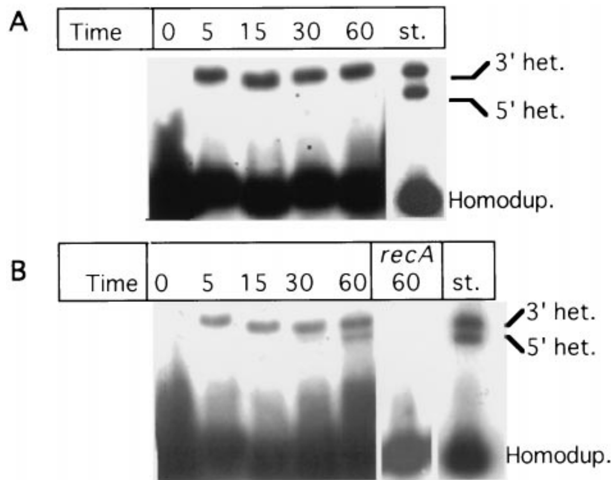


FIG. 3. Heteroduplex production in λZS820-infected *recD* mutants. Equal amounts (10 μg/lane) of DNA from clear lysates (*A*) or total DNA preparations (15 μg/lane) (*B*) were analyzed as described in legend to Fig. 2. A digested sample from an infected *recA recD* mutant is presented as a negative control.

tion. We tested the effect of *xonA* and *recJ* mutations on the rate of accumulation of intramolecular recombination products (Fig. 4) and on the polarity of the strands incorporated into heteroduplex structures (Fig. 5). A role for exonuclease I in recombination is suggested by the observation that a *xonA* mutation lowered recombination proficiency in $recD^+$ cells and in *recD* mutants. On the other hand, a *recJ* mutation lowered recombination proficiency in *recD* mutants but not in $recD^+$ cells. Regardless of the *recD* genotype, intramolecular recombination proficiency in *recJ xonA* double mutants was lower than that in isogenic strains with a single *recJ* or *xonA* mutation (Fig. 4). The dependence of recombination in *recD* mutants on RecJ activity and earlier results (35) suggest that the RecD and RecJ proteins play overlapping roles in the recombination pathway.

To gain insight into the possible role of exonuclease I and RecJ exonuclease in determining heteroduplex strand polarity, we tested the effect of the corresponding mutations on the structure of heteroduplexes incorporated into intramolecular recombination products. Lysogenic *recD* mutants and isogenic derivatives with *xonA2* and *recJ284* mutations were infected by λZS820, and preparations of total cellular DNA from samples taken at time intervals were analyzed for heteroduplex strand polarity. Both *xonA* and *recJ* mutations caused an earlier appearance of the 5′ heteroduplex in total cellular DNA

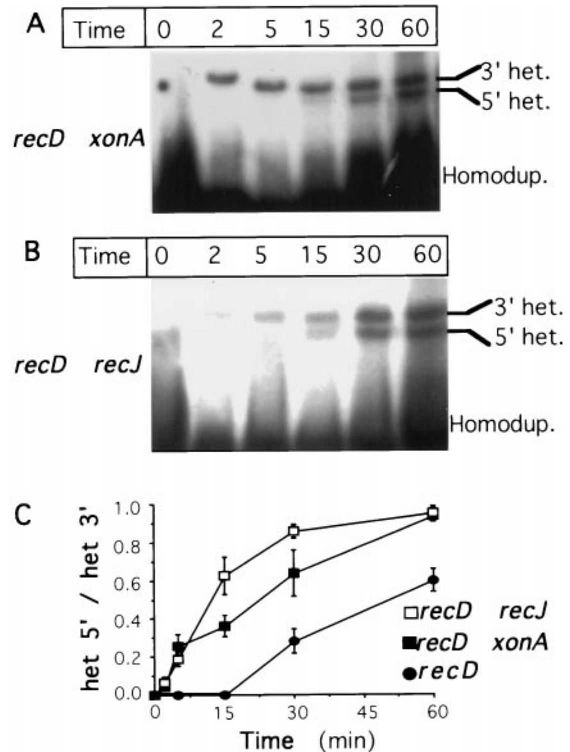
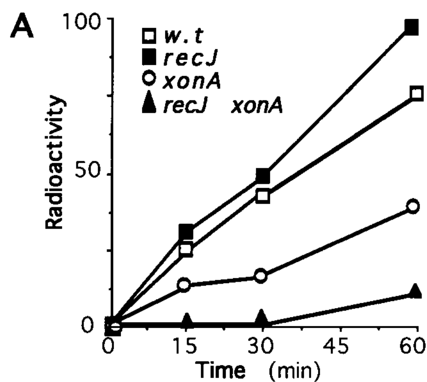


FIG. 5. The effect of *xonA* and *recJ* mutations on the accumulation of heteroduplex products in infected *recD* mutants. Total cellular DNA preparations (15 μg/lane) of samples taken at the indicated times after infection of *recD* mutants and isogenic derivatives with *xonA* (*A*) and *recJ* (*B*) mutations were analyzed for heteroduplex strand polarity as described in the legend to Fig. 2. The ratios of 5′ to 3′ heteroduplex structures in preparations of samples taken from the infected cultures were determined by phosphorimaging analysis (*C*). Presented are data averaged from three determinations for each strain. Datum points are shown with SEs.

preparations and an increase in its relative rate of accumulation (Fig. 5). Furthermore, the 5′ heteroduplex structures were observed also in clear lysates of infected *recJ* and *xonA* mutants (see Fig. 6).

The 5′ Heteroduplex Is Located on a Noncircular Molecule. Because clear lysate preparations of infected *xonA recD* and *recJ recD* mutants contained both 3′ and 5′ heteroduplexes, we examined these preparations, as well as total cellular DNA preparations of infected wild-type cells (Fig. 2), for the distribution of the two heteroduplex types between circular and



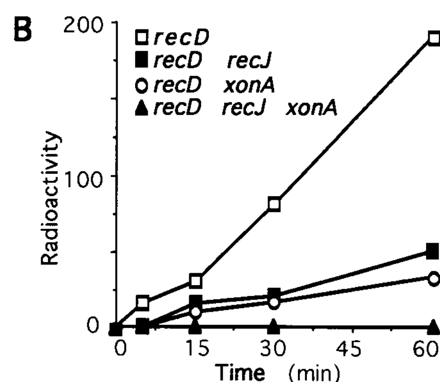


FIG. 4. The effect of *xonA* and *recJ* mutations on the rate of recombination product accumulation in $recD^+$ cells (*A*) and in *recD* mutants (*B*). Strains of the designated genotypes, expressing *Eco*RI from pMB4, were infected by chimeric phage harboring intramolecular recombination substrates. The kinetics of product formation in wild-type cells and the indicated mutants was determined by phosphorimaging analysis of Southern blots as described before (30). Similar effects of the relevant mutations on the kinetics of product formation were observed in five independent experiments.

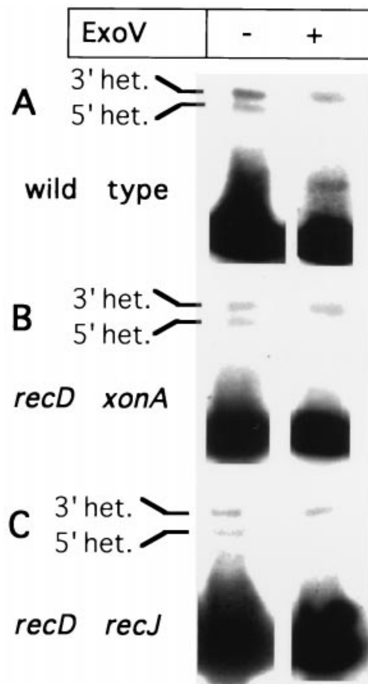


FIG. 6. Heteroduplex susceptibility to RecBCD nuclease digestion. A total cellular DNA preparation of a sample taken at 45 min after infection of wild-type cells (*A*) and clear lysate preparation of samples taken at 30 min after infection of *recD xonA* (*B*) and *recD recJ* (*C*) cells were digested by RecBCD nuclease as described in text. Heteroduplex strand polarity was determined as described in the legend to Fig. 2.

noncircular recombination products. RecBCD enzyme (exonuclease V) degrades linear duplex DNA but not circular duplex DNA. Therefore, this enzyme can be used to eliminate linear DNA from circular DNA preparations (36). To examine the molecular structures containing the 3′ and 5′ heteroduplexes, we tested the susceptibility of the two heteroduplex types to degradation by RecBCD enzyme. Total cellular DNA preparation of a sample taken at 45 min after infection of wild-type cells and clear lysate preparations of samples taken at 30 min after infection of *recD xonA* and *recD recJ* mutants were digested by RecBCD exonuclease. The distributions of heteroduplex types in the digested and nondigested preparations were compared (Fig. 6). Both 3′ and 5′ heteroduplexes were observed in nondigested preparations of all three DNA samples, but only the 3′ heteroduplex survived digestion by RecBCD enzyme. The susceptibility to degradation by RecBCD of the 5′ heteroduplex, but not the 3′ heteroduplex, indicated that only the 3′-ending strand was incorporated into a circular product in the primary pairing reaction. It also suggested that the 5′-ending strand was incorporated into a heteroduplex structure on the reciprocal linear product during branch migration (see Fig. 1).

DISCUSSION

Enzymatic studies of the homologous pairing reaction indicate that the formation of heteroduplex joints in recombination is initiated by invasion of duplex DNA by a single-stranded homologue and proceeds by branch migration of the three-stranded junction. Migration of the junction into a duplex–duplex region generates a four-stranded Holliday intermediate that contains two heteroduplex types. The primary type consists of the invasive strand and its homologue and the reciprocal type of the strands displaced by extension of the primary heteroduplex. In an intramolecular crossover reaction by the substrates used in this study, the primary and reciprocal heteroduplex types are incorporated into circular and linear products, respectively (Fig. 1). Hence, by analyzing the molecular structures containing the two heteroduplex types, we were able to determine the polarity of the invasive strand in the homologous pairing reaction. Because only the 3′ heteroduplex was incorporated into RecBCD-resistant molecules, we conclude that, in *E. coli* recombination, only the 3′-ending strand participates in the primary homologous pairing reaction. The susceptibility of the complementary heteroduplex to degradation by RecBCD (Fig. 6) suggests that the 5′-ending strand is incorporated into a linear recombination product by a process of reciprocal strand exchange.

Different models postulate different roles for RecD at the presynaptic stage of recombination. Whereas some models argue for a Chi-dependent conversion of a RecBCD exonuclease to a RecBC(D) recombinase by ejection or inactivation of RecD (13), others postulate a positive role for RecD in the generation of a ssDNA-ending substrate (12, 37). The observation that, in *recD* mutants, recombination depends on RecJ activity (ref. 35 and this work) supports the latter possibility. We believe that, *in vivo, recD* and recJ products are actively involved in the generation of substrates with a 3′-ending ssDNA overhang for the homologous pairing reaction. We note, however, that the bias for the 3′ heteroduplex structures in circular recombination products was maintained in *recD recJ* double mutants. This result may indicate that pairing of a 3′-ending single strand is an intrinsic property of the cellular homologous pairing mechanism [as seen *in vitro* with purified components (20)] or that a RecD- and recJ-independent mechanism generates homologous pairing substrates with a 3′-ending ssDNA.

recB21 is a polar mutation that also inactivates RecD (38). Because intramolecular recombination by the substrates used in this study is not detectable in *recB21* mutants (30), we conclude that RecB, like RecJ, is required for recombination in $RecD^-$ cells. This dependence suggests that, in *recD* mutants, RecJ 5′-specific exonuclease (39) acts in concert with RecB(C). Assuming that RecBC is involved in similar end-processing mechanisms in *recD* mutants and in $recD^+$ cells, it is conceivable that RecD-dependent recombination in wild-type cells, like RecJ-dependent recombination in *recD* mutants, involves exonucleolytic degradation of the 5′-ending strand. However, our results do not exclude the possibility of different mechanisms for RecJ- and RecD-dependent production of 3′-ending substrates for the RecA-mediated homologous pairing reaction.

Recombination products with 5′ heteroduplex structures accumulated in total cellular DNA preparations at a lower rate than products with 3′ heteroduplex structures, and their accumulation was inhibited by exonucleases that degrade 3′- or 5′-ending ssDNA. These observations may indicate that the generation of 5′ heteroduplex structures is inhibited by ssDNA-specific exonucleases. Alternatively, this result may be explained by the susceptibility of linear DNA to degradation by a *xonA*- and *recJ*-dependent mechanism (29). The first possibility is consistent with the observed dependence of recombination on *xonA* activity. Exonuclease I has been postulated to enhance recombination by generating a substrate with a 5′-ending ssDNA overhang for the homologous pairing reaction or by degrading the displaced strand during branch migration and thereby making strand exchange nonreciprocal (18, 19, 22). Our observation that only the 3′ heteroduplex type is incorporated into circular products of intramolecular recombination and genetic analysis of λ recombination products (40) argues against the initiation of homologous pairing by a 5′-ending single strand. We therefore favor a role for exonuclease I at the branch migration stage of recombination. A similar role has been proposed for RecJ exonuclease (23).

In the presence of ssDNA-binding protein, RecA-mediated pairing is favored at the 3′ end (10, 20), but strand exchange has a 5′→3′ polarity, with respect to the invasive single strand

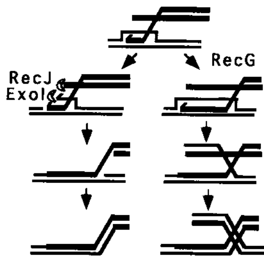


FIG. 7. Alternative mechanism can drive strand exchange and extend heteroduplex structures. Degradation of the displaced strands in branch migration by exonuclease I (ExoI) and/or RecJ nuclease (RecJ) would make strand exchange nonreciprocal and unidirectional (left pathway). Strand exchange, initiated by invasion of a 3′-ending strand, may be driven into duplex–duplex regions by RecG helicase.

(41). Making strand exchange unidirectional by degradation of the displaced strands may help overcome RecA 5′→3′ strand exchange polarity. An alternative mechanism for overcoming RecA 5′→3′ strand exchange polarity may involve RecG-mediated migration of the three-stranded junction into a duplex–duplex region (24, 42). Thus, ssDNA-specific exonucleases and RecG helicase may participate in different but overlapping mechanisms that enhance recombination by making strand exchange unidirectional (Fig. 7).

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