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Genetics

The recombination hot spot χ activates RecBCD recombination by converting *Escherichia coli* to a *recD* mutant phenocopy

(genetic recombination/exonuclease V/bacteriophage λ)

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ABSTRACT **The products of the *recB* and *recC* genes are necessary for conjugal recombination and for repair of chromosomal double-chain breaks in *Escherichia coli*. The *recD* gene product combines with the RecB and RecC proteins to comprise RecBCD enzyme but is required for neither recombination nor repair. On the contrary, RecBCD enzyme is an exonuclease that inhibits recombination by destroying linear DNA. The RecD ejection model proposes that RecBCD enzyme enters a DNA duplex at a double-chain end and travels destructively until it encounters the recombination hot spot sequence χ. χ then alters the RecBCD enzyme by weakening the affinity of the RecD subunit for the RecBC heterodimer. With the loss of the RecD subunit, the resulting protein, RecBC(D^-), becomes deficient for exonuclease activity and proficient as a recombinagenic helicase. To test the model, genetic crosses between λ phage were conducted in cells containing χ on a nonhomologous plasmid. Upon delivering a double-chain break to the plasmid, λ recombined as if the cells had become *recD* mutants. The ability of χ to alter λ recombination in trans was reversed by overproducing the RecD subunit. These results indicate that χ can influence a recombination act without directly participating in it.**

In infections of *Escherichia coli* by phage λ mutant for the *red* and *gam* genes, RecBCD enzyme (exonuclease V; exoV) can enter a λ genome that has been linearized at *cos*, the site of action of terminase (Ter) (1). RecBCD enters primarily at the right end of linearized λ since the left end remains bound by Ter (2, 3). Other entry sites are provided by λ DNA replication (4, 5). In the absence of χ, an octanucleotide (6) hot spot for RecBCD-promoted recombination (7), RecBCD-catalyzed exchanges are distributed approximately uniformly along the length of nonreplicated λ chromosomes (8). In the presence of a properly oriented χ (9–11), exchange is stimulated at χ and decreases in a gradient extending leftward of χ (7, 12).

RecBCD is both a χ-stimulated recombinase and a potent exonuclease. Mutations in the *E. coli recB* and *recC* genes eliminate all activities of RecBCD; such mutants are $exoV^-$ and Rec^-. In contrast, *recD* mutants are defective in exoV activity but catalyze recombination at high rates (13, 14). Exchanges in *recD* cells occur at the rate observed for χ-stimulated recombination in rec^+ hosts (13) but take on a unique distribution: exchanges are neither uniform along the chromosome nor stimulated at χ but instead are focused at DNA ends (15). Among phage with unreplicated chromosomes, in which double-chain breaks occur only at *cos*, recombination in *recD* cells is focused at the right end of the λ map. *recD* mutants appear to be constitutively activated for recombination (16).

The exoV activity of RecBCD is attenuated by χ—linear DNA containing a χ site is protected from degradation (3, 17, 18). This attenuation protects other linear DNA molecules in the same cell (3). The RecD ejection model proposes that attenuation of RecBCD ($exoV^+$) by χ involves loss or modification of the RecD subunit resulting in RecBC(D^-) ($exoV^-$) (15, 19). To determine whether χ-attenuated RecBCD enzyme is recombination deficient (making the cell resemble a *recC* or *recB* null mutant) or is recombination proficient (making the cell resemble a *recD* null mutant), we tested whether (*i*) χ on a heterologous plasmid can act in trans to diminish χ activity in λ × λ lytic crosses, (*ii*) any decrease in χ activity is unaccompanied by a decrease in the overall rate of recombination, (*iii*) providing χ in trans alters the distribution of recombination events, especially in unreplicated phage, and (*iv*) overproduction of the RecD subunit reverses the effect of χ in trans.

MATERIALS AND METHODS

Bacteria and Phage. Bacteria (Table 1) were usually grown on LB medium (20). When bacteria contained plasmids, ampicillin was included at 100 μg/ml or chloramphenicol was included at 10 μg/ml.

Phage λ (Table 1) stocks were prepared as plate lysates (22). For physical studies of cosmid linearization by λ, λ stocks were purified by CsCl density gradient centrifugation followed by dialysis against 10 mM Tris·HCl (pH 7.4) containing 10 mM $MgSO_4$. For crosses executed under conditions of limited DNA synthesis, "heavy" phage stocks were prepared on a modified M9 medium (22) supplemented with 0.65% agar containing [^{13}C]glucose and [^{15}N]ammonia.

Recombination of Replicated and Unreplicated λ Chromosomes. Crosses of density-labeled phage were performed in homoimmune lysogens to limit DNA replication (23). Heavy phage (five per cell of each genotype) and light heteroimmune helper phage MMS444 (three per cell) were adsorbed to lysogens as described (22). Cleared lysates were brought to an index of refraction of 1.378 with cesium formate and centrifuged to near equilibrium; the resulting density gradients were collected as two-drop fractions.

Time Course of Cosmid and λ DNA Linearization by Ter. AK24 (pK11) cultures were infected with MMS517 at the indicated multiplicity of infection (moi) essentially as described (22). Phage were adsorbed on ice for 15 min, transferred to a 37°C water bath, and incubated for 5 min with gentle stirring. The mixtures were introduced into 9 ml of prewarmed LB medium and incubated at 37°C with shaking; 1.5-ml aliquots were withdrawn at intervals to purify the total intracellular DNA (3). Samples were digested with *Bst*EII, heated at 70°C for 20 min, and electrophoresed in agarose gels. After electrophoresis, gels were treated and transferred as described (3). Radioactive probes (*Eco*RI-linearized DNA of pK22 or *Bst*EII-cut λ) were prepared by random-primer labeling. Prehybridization, hybridization, and autoradiography were as described (3). DNA species were quantified by direct radioactivity scanning of the membrane by Ambis Systems.

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Abbreviations: exoV, exonuclease V; Ter, terminase; moi, multiplicity of infection.

Table 1. Strain list

	Genotype or description	Source*
E. coli K-12		
594	*galK2 galT22 rpsL179 IN(rrnD-rrnE)1*	J. Weigle
AK24	JC5220 SuII Δ(*srlR-RecA*)*304 recD1903*::Tn*10dTet*	This paper
FS1576	SuII *thi thr leu recD1009*	
FS3680	594 *hsdR* (λ MMS441)	This paper
JC5220	*thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	A. J. Clark
Plasmid		
pB100	RecD overexpressing plasmid, *colE1* replicon	(21)
pK11	Cosmid, p15A replicon	(3)
pK17	pK11 with 3 actively oriented χ sites	This paper
pK20	pK17 with *cos* deleted	This paper
pK22	Cosmid, p15A replicon	(3)

	Genotype or description	Source*
Phage λ		
JMC177	*Jts15 int4 red3 gam210*	
JMC241	*int4 χB121 red3 gam210 cI857 Rsus5*	
MMS441	*Jts15 int4 red3 gam210 Rsus5*	
MMS444	*Jts15 int4 red3 gam210 imm*^{434} *Rsus5*	
MMS517	*red3 gam210 cI26 χD123*	
MMS555	*Jsus6 b1453 cI857 χD123*	
MMS556	*b1453 χD123 Rsus5*	
MMS557	*Jsus6 b1453 χ76 cI857*	
MMS558	*b1453 χ76 Rsus5*	
MMS1816	*Jts15 int4 red3 gam210 cI857 nin5*	
MMS1817	*int4 red3 gam210 nin5 Ssus7*	
MMS2076	*Jts15 red3 gam210 nin5 Ssus7*	
MMS2084	*Jts15 red3 gam210 imm*^{434} *nin5 Ssus7*	
REM266	*Jsus6 b1453 cI857*	
REM272	*b1453 Rsus5*	
REM274	*int4 red3 gam210 cI26 Rsus5*	
REM275	*int4 red3 gam210 cI26 χD123 Rsus5*	

*Lab collection unless otherwise indicated.

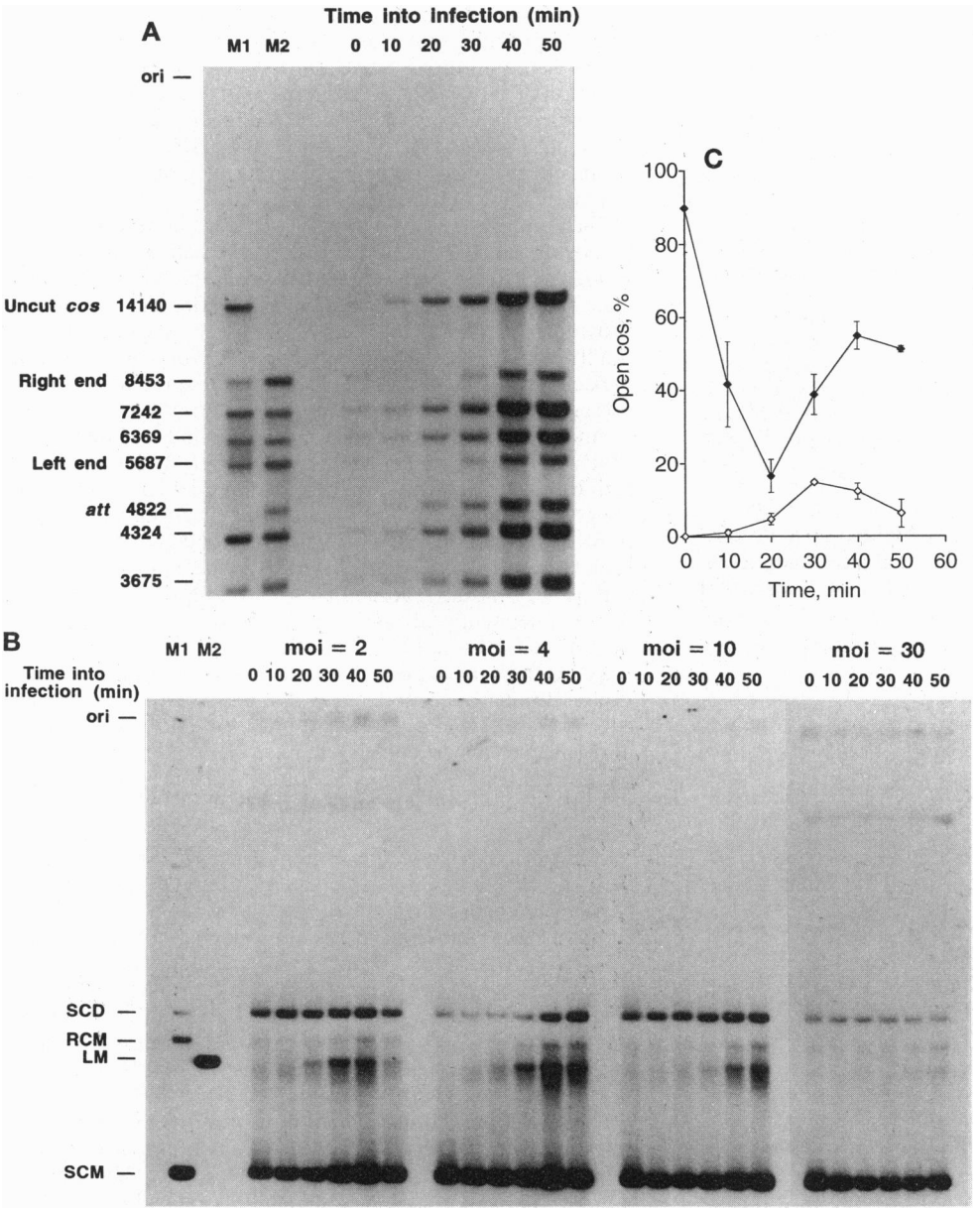

FIG. 1. Linearization of λ and cosmid DNA by λ Ter. (*A*) Kinetics of λ *cos* cleavage (moi = 3). ori, Origin of the gel; M1, ligated λ DNA digested with *Bst*EII; M2, λ DNA digested with *Bst*EII. Lengths (bp) of *Bst*EII-generated fragments are provided. Uncut *cos*, the right and left ends of λ DNA linearized at *cos*, and the *att*-containing *Bst*EII fragment are marked. The *att*-containing fragment is underrepresented in our preparations, possibly because of an Int-mediated unsealable double-strand scission at *att*. (*B*) Kinetics and efficiency of cosmid cleavage, with moi as indicated. ori, Origin of the gel; M1, cosmid DNA preparation containing supercoiled monomer (SCM), relaxed circular monomer (RCM), and supercoiled dimer (SCD) species; M2, linearized monomer cosmid DNA (LM). (*C*) Time course of cosmid (◇) and λ (◆) DNA linearization by Ter. Data are means (±SD) of four independent experiments (moi = 1–3).

Table 2. Providing χ in trans

Rec	Cosmid	λ-borne χ	Total yield/10^5	% Rec	c/+	χ activity
			Series A			
+	pK11	χ76	170	2.6	9.6	5.7
+	pK11	χD	430	2.0	0.33	
+	pK17	χ76	190	2.9	6.0	4.3
+	pK17	χD	260	3.2	0.32	
			Series B			
+	pK11	χ76	62	2.2	12	6.0
+	pK11	χD	120	3.9	0.33	
+	pK17	χ76	270	4.8	2.6	2.6
+	pK17	χD	360	4.2	0.39	
			Series C			
+	pK11	No χ	2.0	2.9	1.8	
+	pK11	χ76	47	5.7	12	5.9
+	pK11	χD	57	6.1	0.34	
+	pK17	No χ	4.4	3.1	1.2	
+	pK17	χ76	42	5.5	5.7	4.3
+	pK17	χD	47	6.3	0.3	
			Series D			
recB21		No χ	20	1.0	0.90	
recB21		χ76	15	0.52	0.88	1.2
recB21		χD	15	0.53	0.86	
recD1014		No χ	130	5.1	0.93	
recD1014		χ76	240	4.9	0.91	1.1
recD1014		χD	240	4.5	0.74	
rec$^+$	pK11					5.8 (0.2)*
rec$^+$	pK17					4.3 (0.1)*

λ lytic crosses (moi = 10) were performed essentially as described (23). Total yields of phage in the cross lysates were determined by plating on FS1576 at 34°C. Yields of recombinant phage were determined by plating on 594 *recD* at 42°C. Recombination between *J* and *cI* yielded recombinants that produced clear plaques. χ activity was determined as described (23). MMS558 × MMS557 examined the activity of χ76; MMS556 × MMS555 examined the activity of χD; REM272 × REM266 monitored λ crosses without λ-borne χ sites. All crosses were in *hsdR* derivatives of *E. coli* 594 except series B, which were in the *E. coli* JC5220 background. Means ± SD of χ activity in 594 *hsdR* were determined from values shown in series A and C and two other series. Rec, *E. coli* recombination genotype; % Rec, recombinant yield/total yield) × 100%.
*Mean χ activity (SD); $n = 4$.

Construction of pK17 and pK20 Plasmids. To construct pK17, three χ-containing self-complementary oligonucleotides were inserted into pK11 (3) at the *Kpn* I, *Sal* I, and *Bam*HI restriction sites of that plasmid to create the following sequence (χ sites are boldface; relevant restriction sites are underlined):

GAATTCGAGCTCGGTACCACCA**GCTGGTGG**TACCCGGGGATCCACCA-
*Eco*RI *Kpn* I *Kpn* I *Bam*HI

GCTGGTGGATCCTCTAGAGTCGACCACCA**GCTGGTGG**TCGACCTGCAG-
*Bam*HI *Sal* I *Sal* I *Pst* I

---*cos*---CTGCAGGCATGCAAGCTT
(450 bp) *Pst* I *Hin*dIII

This sequence contains three χ sites in each orientation. pK20 was constructed from pK17 by removing the *Pst* I/*Pst* I *cos*-containing fragment.

RESULTS

Cosmid Linearization by λ-Produced Ter. To measure the extent of cosmid linearization by λ and to determine optimal conditions for linearization of λ and cosmids, *E. coli* cells harboring a χ-less cosmid (pK11) were infected at different moi with λ *red gam*, and total DNA was purified from infected cells at different times. A *recA recD* strain (AK24) was used because *recD* mutations inactivate exoV, which would otherwise degrade linearized cosmid DNA (3), while *recA* mutations prevent the accumulation of linear plasmid multimers in *recD* mutants (unpublished data).

λ linearization was measured by restricting total DNA with *Bst*EII and monitoring interconversion of an uncut *cos*-containing DNA fragment and the two products of *cos* cutting. Upon infection of *E. coli*, λ injects its linear chromosome. At time 0 after infection, 90% of injected λ chromosomes remain open at *cos* (Fig. 1*A* and *C*). Linear λ chromosomes circularize by annealing and ligation of *cos*. By 20 min, the fraction of open λ chromosomes drops to <20% and subsequently begins to increase again (presumably due to production of Ter) reaching a value of >55% by 40 min (Fig. 1*C*), at which time cell lysis begins.

In the same DNA preparations, cosmid cutting parallels λ cutting (Fig. 1*C*). The first signs of cosmid DNA linearization appear at 20 min, with the peak of linearization between 30 and 40 min (Fig. 1*C*). Cosmid linearization is ≈15% for moi up to 10 and is lower at moi >20 (Fig. 1*B*).

χ Acts in Trans to Diminish the χ Dependence of RecBCD Recombination. RecBCD interacts with χ only if a χ-containing DNA molecule is linear and has an unblocked end (3, 4, 24). To provide χ in trans, we introduced a small circular plasmid containing *cos* and three χ sites into cross hosts. After infection of these cells by λ, the plasmid is linearized by λ Ter (see above). In this way, χ is provided in trans at the same moment that λ becomes accessible to RecBCD. This simultaneous unmasking of many χ sites in trans permits RecBCD to interact with χ prior to interacting with λ.

Phage were crossed in Rec$^+$ *E. coli* containing pK17, harboring three χ sites, and in isogenic cells containing the χ-free control cosmid pK11. χ activity in these freely replicating λ lytic crosses was determined as described (23) (Table 2). Providing χ in trans decreased χ activity; the decrease was significant ($P < 0.05$ by χ^2 analysis) in every pairwise comparison of crosses in the presence of pK17 with crosses in the presence of pK11 in both strain backgrounds tested.

Since χ is specific for RecBCD (23), a χ-induced decrease in χ activity in λ lytic crosses indicates that RecBCD enzyme activity is altered by χ on the cosmid. The yield of recombinant phage from crosses in pK17 hosts remains as high as the yield in pK11 crosses, indicating that the altered RecBCD activity retains recombination proficiency. By decreasing χ activity without decreasing recombination rates, pK17 makes *E. coli* a partial phenocopy of *recD* mutant cells rather than *recB/C* mutant cells (Table 2).

χ Acts in Trans to Focus RecBCD Recombination at Double-Chain Breaks. Density-labeled λ phage were crossed under conditions of restricted DNA replication and separated into unreplicated (two heavy DNA chains, HH) and replicated (one or two light DNA chains, HL or LL, respectively) fractions (25). Recombinants that enjoyed an exchange near *cos* can be distinguished from recombinants that crossed over far from *cos* by following segregation of a clear plaque morphology mutation.

In the absence of χ on the cosmid (pK11), recombination frequently occurs far from *cos* in all fractions (Fig. 2*A*). In contrast, recombination in the presence of χ on the cosmid (pK17) is focused near *cos* in the unreplicated phage peak and becomes frequent far from *cos* only in the replicated λ peaks (Fig. 2*B*). Therefore, RecBCD recombination that is χ-transactivated by pK17 (Fig. 2*B* and Table 3) manifests the crossover distribution of RecBC(D$^-$) recombination that occurs in *recD* mutants (Fig. 2*D* and Table 3) (15).

Substituting pK20, a derivative of pK17 that is deleted for *cos* but retains the multiple χ sites, eliminates transactivation (Fig. 2*C* and Table 3). χ-transactivated RecBCD recombination differs from χ-cisactivated RecBCD recombination (e.g.,

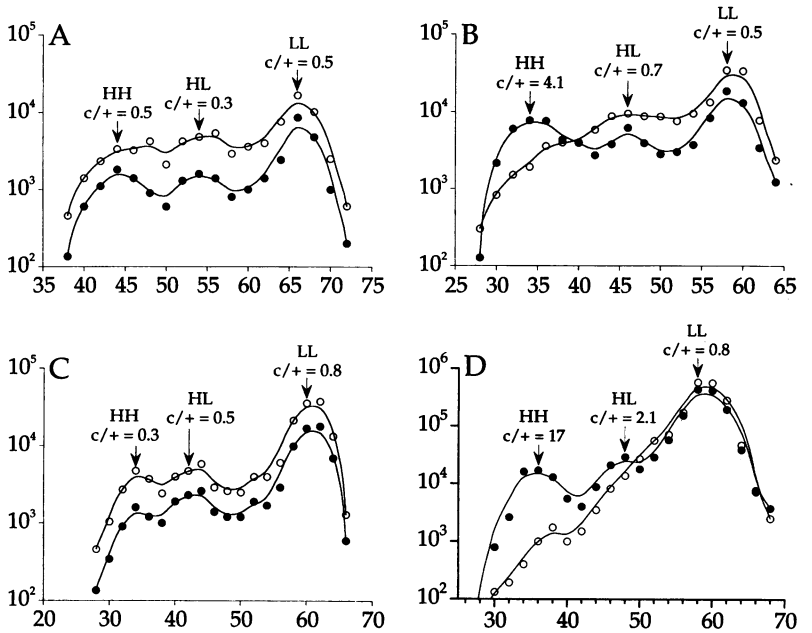

FIG. 2. Recombination of replicated and unreplicated chromosomes. Yields of clear (●, *cos* proximal) and turbid (○, *cos* distal) recombinant phage plotted against fraction number are shown. Unreplicated (HH) and replicated (HL and LL) peaks are identified, and the ratio of *cos*-proximal/*cos*-distal recombinants in the median fraction of each peak is indicated with an arrow. Each panel depicts a cross of REM274 × JMC177 plated on 594 *recD*(λ MMS444) at 42°C to select for J^+ R^+ recombinants. Phage do not contain χ. (*A*) Gradient 1 (see Table 3) from FS3680 (pK11). (*B*) Gradient 1 from FS3680 (pK17). (*C*) A FS3680 (pK20) gradient. (*D*) A FS3680 *recD1903*::Tn*10dTet* gradient.

exchanges stimulated by χD or χB in λ). Cis-activated crossovers are frequently near χ without regard to chromosome replication—i.e., to the position of the double-chain end that admits RecBCD (see χD and χB crosses; Table 3). As in the freely replicating crosses, providing χ in trans on pK17 decreases the activity of both χD and χB. χ-transactivated RecBCD recombination is independent of *recR* and *recJ* gene activities (Table 3) and therefore is not attributable to the RecF pathway (27, 28).

Overproduction of RecD Inhibits Transactivation of RecBCD by χ. Since transactivated RecBCD recombination resembles recombination in *recD* cells, we surmised that transactivated RecBCD had either lost or inactivated the RecD subunit. We repeated the crosses outlined in Fig. 2 in cells that overproduced the RecD subunit. Overproduction of RecD returns the exchange distribution to that observed in the absence of χ (Table 3). In addition, overproduction of RecD inhibits RecBCD recombination, resulting in low yields of unreplicated phage (Fig. 3).

DISCUSSION

We conducted genetic crosses between λ phage in cells containing χ in trans on a nonhomologous plasmid. Delivery of a double-chain break to the χ-bearing cosmid decreased the χ-dependence of λ recombination and focused λ recombina-

Table 3. χ transactivates RecBCD recombination by making cells RecD⁻

Relevant *E. coli* genotype	Relevant λ genotype	*cos* proximal/*cos* distal		
		HH	HL	LL
rec⁺ (pK11)		0.5	0.3	0.5
		0.6	0.4	0.4
rec⁺ (pK17)		4.1	0.7	0.5
		3.3	0.5	0.6
rec⁺ (pK20)		0.3	0.5	0.8
recD1903::Tn*10dTet*		17	2.1	0.8
rec⁺	χD	6.0	5.1	7.8
rec⁺ (pK17)	χD	7.8	3.5	4.1
rec⁺ (pK11)	χB	0.078	0.049	0.063
rec⁺ (pK17)	χB	0.104	0.066	0.085
recR1502::Tn*10dKan* (pK11)	*nin5*	0.5	0.4	0.5
recR1502::Tn*10dKan* (pK17)	*nin5*	4.1	0.5	0.6
recJ284::Tn*10* (pK17)		9.2	2.0	0.6
rec⁺ (pK11) (pB100)		0.5	0.4	0.4
		0.6	0.4	0.4
rec⁺ (pK17) (pB100)		0.5	0.4	0.4
		0.5	0.3	0.4

Crosses were performed as described in Fig. 2 in derivatives of FS3680 with the following additions: (*i*) χD crosses used REM275 in place of REM274; χB crosses replaced REM274 with JMC241; (*ii*) crosses performed in *recR* mutant cells used phage deleted for the λ *recR* analog *orf* (26) (MMS1816 × MMS1817 with MMS2084 provided as helper in strains bearing MMS2076 as a prophage) selecting for J^+ S^+ recombinants; (*iii*) to overexpress RecD from pB100 (21), isopropyl β-D-thiogalactopyranoside was added to 1 mM 15 min before infection by λ. RecD overexpression was verified by SDS/PAGE of whole cell lysates (20) (data not shown). For all crosses, the unreplicated (HH) and replicated (HL and LL) peaks are identified as in Fig. 2, and the ratio of the titer of recombinants that crossed over to the right of *c*I to the titer of recombinants that crossed over to the left of *c*I in the median fraction of each peak is indicated as *cos* proximal/*cos* distal.

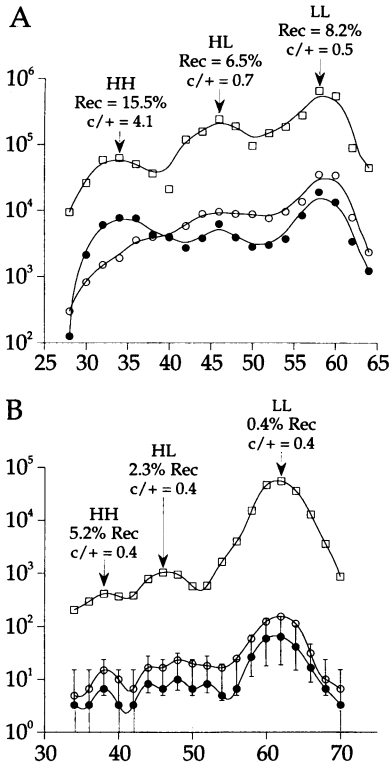

FIG. 3. Recombination (as in Fig. 2) in the presence of excess RecD. Total phage (□) are plotted separately. Percentage recombination in the median fraction of each peak is indicated with an arrow. Each vertical bar in *B* indicates 95% confidence interval for the titer of clear plaque-forming phage. (*A*) Gradient 1 (see Table 3) from FS3680 (pK17). (*B*) Gradient 1 (see Table 3) from FS3680 (pK17) (pB100).

tion to the double-chain break site, *cos*, mimicking the exchange patterns seen in λ crosses conducted in *recD* mutant cells. Overproduction of RecD protein in these host cells restored the distribution of exchanges to wild type. These results indicate that χ is trans dominant and can influence a recombination act without directly participating in it, consistent with the model that χ activates RecBCD recombination by ejecting or altering the RecD subunit of RecBCD enzyme (15, 19, 29).

The paradox of an enzyme that destroys its substrate, linear DNA, being required to recombine that same substrate is resolved by recognizing that χ acts as a molecular switch that toggles RecBCD nuclease to RecBC(D^-) recombinase (15, 16, 19, 26, 29–31). Since χ need not be in cis to stimulate RecBCD recombination, it is economical to propose that the only role of χ is to convert RecBCD enzyme from a destructive nuclease to a productive helicase (16, 30). According to this view, χ hot spot activity reflects the preference of RecBC(D^-) to promote recombination at the first opportunity for pairing, usually adjacent to the χ site that activated RecBCD (R.S.M., M. M. Stahl, and F.W.S., unpublished data). For the relevance of these data to other models for the role of χ in recombination, see ref. 16.

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1. Kobayashi, I., Murialdo, H., Crasemann, J. M., Stahl, M. M. & Stahl, F. W. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5981–5985.
2. Kobayashi, I., Stahl, M. M. & Stahl, F. W. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49,** 497–506.
3. Kuzminov, A., Schabtach, E. & Stahl, F. W. (1994) *EMBO J.* **13,** 2764–2776.
4. Stahl, M. M., Kobayashi, I., Stahl, F. W. & Huntington, S. K. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2310–2313.
5. Stahl, F. W., Kobayashi, I. & Stahl, M. M. (1983) in *Mechanisms of DNA Replication and Recombination*, ed. Cozzarelli, N. (Liss, New York), pp. 773–783.
6. Smith, G. R., Kunes, S. M., Schultz, D. W., Taylor, A. & Triman, K. L. (1981) *Cell* **24,** 429–436.
7. Lam, S. T., Stahl, M. M., McMilin, K. D. & Stahl, F. W. (1974) *Genetics* **77,** 425–433.
8. Stahl, F. W., McMilin, K. D., Stahl, M. M., Crasemann, J. M. & Lam, S. (1974) *Genetics* **77,** 395–408.
9. Faulds, D., Dower, N. & Stahl, M. M. (1979) *J. Mol. Biol.* **131,** 681–695.
10. Stahl, F. W., Stahl, M. M., Malone, R. & Crasemann, J. (1980) *Genetics* **94,** 235–248.
11. Yagil, E., Dower, N. A., Chattoraj, D., Stahl, M., Pierson, C. & Stahl, F. W. (1980) *Genetics* **96,** 43–57.
12. Cheng, K. C. & Smith, G. R. (1989) *Genetics* **123,** 5–17.
13. Chaudhury, A. M. & Smith, G. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7850–7854.
14. Amundsen, S. K., Taylor, A. F., Chaudhury, A. M. & Smith, G. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5558–5562.
15. Thaler, D. S., Sampson, E., Siddiqi, I., Rosenberg, S. M., Thomason, L. C., Stahl, F. W. & Stahl, M. M. (1989) *Genome* **31,** 53–67.
16. Myers, R. S. & Stahl, F. W. (1994) *Annu. Rev. Genet.* **28,** 49–70.
17. Dabert, P., Ehrlich, S. D. & Gruss, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12073–12077.
18. Dixon, D. A. & Kowalczykowski, S. C. (1993) *Cell* **73,** 87–96.
19. Thaler, D. S., Sampson, E., Siddiqi, I., Rosenberg, S. M., Stahl, F. W. & Stahl, M. M. (1988) in *Mechanisms and Consequences of DNA Damage Processing*, eds. Friedberg, E. & Hanawalt, P. (Liss, New York), pp. 413–422.
20. Maloy, S. R. (1990) *Experimental Techniques in Bacterial Genetics* (Jones & Bartlett, Boston).
21. Boehmer, P. E. & Emmerson, P. T. (1991) *Gene* **102,** 1–6.
22. Arber, W., Enquist, L., Hohn, B., Murray, N. & Murray, K. (1983) in *Lambda II*, eds. Hendrix, R. W., Roberts, J. W., Stahl, F. W. & Weisberg, R. A. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 433–466.
23. Stahl, F. W. & Stahl, M. M. (1977) *Genetics* **86,** 715–725.
24. Kobayashi, I., Stahl, M. M., Fairfield, F. & Stahl, F. W. (1984) *Genetics* **108,** 773–794.
25. Stahl, F. W. & Stahl, M. M. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 443–453.
26. Stahl, F. W., Thomason, L. C., Siddiqi, I. & Stahl, M. M. (1990) *Genetics* **126,** 519–533.
27. Clark, A. J. (1973) *Annu. Rev. Genet.* **7,** 67–86.
28. Sawitzke, J. A. & Stahl, F. W. (1992) *Genetics* **130,** 7–16.
29. Dixon, D. A., Churchill, J. J. & Kowalczykowski, S. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2980–2984.
30. Rosenberg, S. M. & Hastings, P. J. (1991) *Biochimie* **73,** 385–397.
31. Dixon, D. A. & Kowalczykowski, S. C. (1991) *Cell* **66,** 361–371.