χ Recombination Activity in Phage λ Decays as a Function of Genetic Distance

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Manuscript received May 2, 1995
Accepted for publication July 26, 1995

ABSTRACT

In *Escherichia coli*, χ is a recombination hotspot that stimulates RecBCD-dependent exchange at and to one side of itself. χ activity is highest at χ and decreases with distance from χ. The decrease in χ activity may be a simple property of the physical distance over which χ can stimulate recombination. Alternatively, the decay in χ activity with distance may reflect the high likelihood that χ-stimulated recombination occurs in a single χ-proximal act, to the exclusion of additional χ-stimulated exchanges more distal to χ. To test the models, we determined if χ activity decreases as a function of physical distance (*i.e.*, DNA base pairs) or genetic distance (homologous DNA base pairs). Our results indicate that χ activity decays as a function of genetic distance. In addition, we found that the *sbcB* gene product (exonuclease I, a 3′ → 5′ ssDNA exonuclease) modulates the distance over which χ can act. In contrast, the *recJ* gene product (a 5′ → 3′ ssDNA exonuclease) does not alter the decay of χ activity.

SPECIAL sites involved in generalized recombination have been identified in a variety of organisms (*e.g.*, LISSOUBA and RIZET 1960; MURRAY 1963; ANGEL *et al.* 1970; GUTZ 1971; LAM *et al.* 1974; FOGEL *et al.* 1978). These sites are associated with high rates of genetic exchange that diminish with distance from the site (hotspot). In *Escherichia coli* χ is such a hotspot for recombination (reviewed in MYERS and STAHL 1994).

χ hotspot activity depends on RecBCD enzyme, a potent dsDNA exonuclease (exo V) that degrades linear DNA from double-chain-break (DCB) sites up to χ (STAHL *et al.* 1990; DIXON and KOWALCZYKOWSKI 1993). Upon interacting with χ, RecBCD enzyme activity is altered; exo V is inactivated, but the enzyme retains DNA helicase activity (DIXON and KOWALCZYKOWSKI 1993; DIXON *et al.* 1994). This property is shared by *recD* null mutants; RecBC enzyme is exo V$^-$ but retains DNA helicase activity (CHAUDHURY and SMITH 1985; RINKEN *et al.* 1992; DIXON *et al.* 1994). By a number of criteria, *recD* mutants behave as if RecBC enzyme were constitutively activated for recombination (reviewed in MYERS and STAHL 1994). It has been proposed that the conversion of RecBCD enzyme from a destructive exonuclease (exo V) (THALER *et al.* 1988) to a recombinagenic DNA helicase (RecBC) (ROSENBERG and HASTINGS 1991) is the function of χ in homologous recombination. In support of this view, χ has been shown to transactivate RecBCD-mediated recombination (KÖPPEN *et al.* 1995; MYERS *et al.* 1995). Once transactivated, recombination proceeds as if the cells were transiently RecD$^-$ (KÖPPEN *et al.* 1995; MYERS *et al.* 1995).

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Since χ is able to activate RecBCD recombination without directly participating in genetic exchange, why should DNA sequences close to χ experience higher exchange rates than sequences distant from χ? What makes χ a recombination hotspot? Two general classes of models can account for χ hotspot behavior. Model I: The decrease in χ activity is simply a function of the physical distance (in DNA base pairs) from χ as if the χ-activated enzyme were subject to spontaneous dissociation from its substrate or to deactivation by restoration of the RecD subunit. Model II: The decrease in χ activity with distance reflects the high likelihood that χ-stimulated recombination occurs as a single act of exchange close to χ, to the exclusion of additional χ-stimulated exchanges distal to χ. The models are diagrammed in Figure 1.

The two models make different predictions as to how DNA sequence heterology (which blocks RecA-mediated DNA pairing but not RecBC-mediated DNA unwinding) will affect χ recombination hotspot activity. Increasing the length of a heterologous DNA segment immediately to the left of χ increases the distance over which RecBC must travel and unwind DNA before RecA can promote recombination with the subsequent homologous DNA segment. We test the models by requiring χ-activated RecBCD to act across heterologies of known and varied length, measuring χ-stimulated exchange in fixed intervals to the left (interval I) and to the right (interval II) of χ, respectively, and taking the ratio of the two exchange rates. If we view the process as it is diagrammed in Figure 2, Model I predicts the ratio interval I/interval II will decrease as the size of the DNA sequence heterology over which χ must act increases, while Model II predicts that the ratio will not change when the size of the heterology is changed.

MATERIALS AND METHODS

Bacteria: Crosses with *Eco*K-modified phage were conducted in Su$^-$ derivatives of AB1157 (BACHMAN 1987): JC11450 is *rec*$^+$;

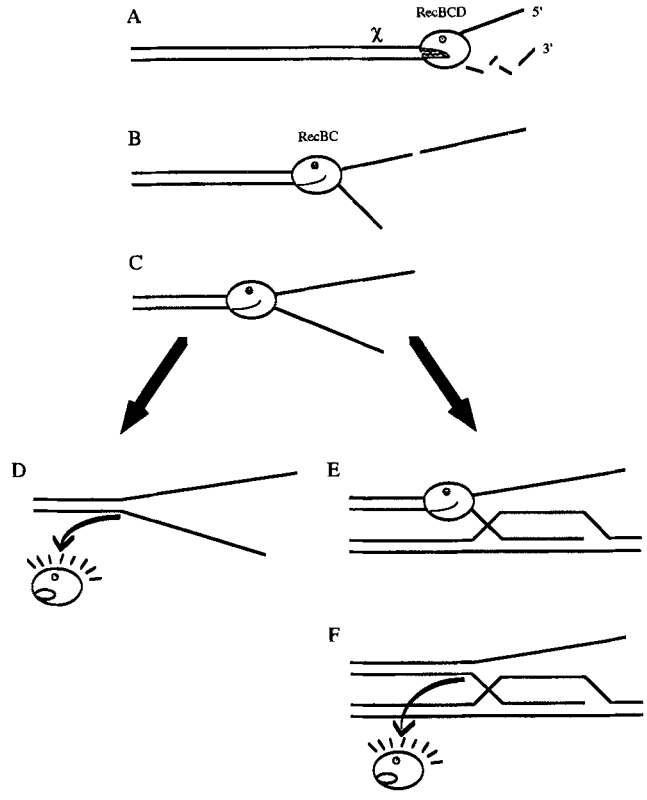

FIGURE 1.—Two scenarios are presented for the decay of χ activity. RecBCD protein gains entry to a DNA duplex at a DCB. DNA is degraded from the point of entry to χ, primarily on the chain ending 3′ at the DCB (A). Recognition of χ converts RecBCD from exo V to RecBC helicase (B). RecBC unwinds DNA beyond χ until it dissociates from the unwound duplex (C and D). If RecBC dissociates from DNA with a rate that is independent of the number of DNA base pairs traveled, the decay of χ activity may reflect the tendency of RecBC to disengage before catalyzing recombination. In this scenario, χ activity decreases as an exponential function of the number of DNA base pairs traversed by RecBC following χ recognition. Alternatively, RecBC may travel in a highly processive manner until it promotes a single act of recombination (E). In this alternative scenario, χ activity is maintained at full potential until DNA unwound by RecBC engages a homologous DNA duplex (aided by RecA protein). Exchange is proposed to extinguish χ activity. We offer two possible mechanisms for the limitation of RecBC to one shot at recombination. The first proposes that the tips of unwound DNA are required for recombination, and once used (that is, repaired) the resulting product is no longer recombinationally active to the left of χ (as drawn). The second proposes that following a productive RecA-mediated pairing event, RecBC falls off, or is removed, from the unwound chromosome (F) limiting the extent of unwinding beyond the paired region. The model is adapted from that of ROSENBERG and HASTINGS (1991).

FS2103 is *recJ284*::Tn*10*; FS2203 is *sbcB15*; FS2505 is *sbcB15 recJ284*::Tn*10*; FS3688 is *sbcB15 recJ2001*::Tn*10dKn*; FS3831 is *recJ2001*::Tn*10dKn*; FS3829 is *sbcB15 dnaBts22 malB*::Tn*9*. Some crosses were conducted with unmodified phage in JAS13 (*galK2 galT22 rpsL179 hsdR*(r^- m^+) IN(*rrnD-rrnE*)*1*) and FS2944 (as JAS13 but *dnaBts22 malB*::Tn*9*). Particles that infect JAS13 or FS2944 become modified for *Eco*K and subsequently escape restriction when plated on restricting hosts.

λ: All phage genotypes (Figure 3) were constructed in this laboratory in standard or UV-stimulated lytic crosses. λ genetic elements are defined in Table 1. The positions of deletion breakpoints in the λ genome, taken from DANIELS *et al.* (1983), are summarized in Table 2. Revertant frequencies of the *Rsus5* and *Asus32* alleles in our single-mutant stocks were low ($\leq 3 \times 10^{-5}$ and $\leq 4 \times 10^{-6}$, respectively), so that revertant phage did not contribute significantly to the estimate of the recombinant titer.

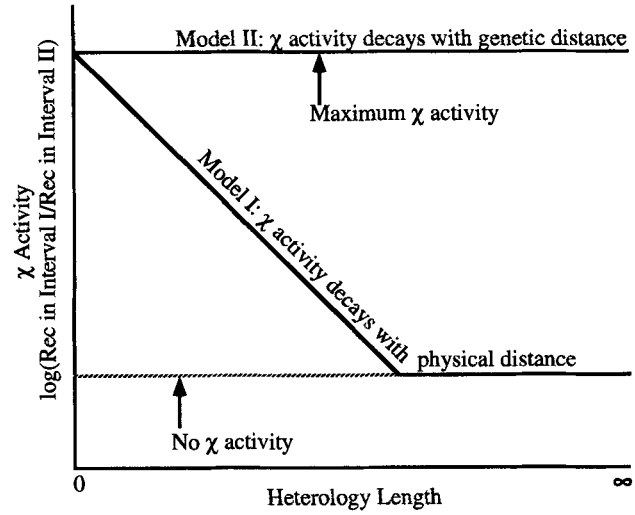

FIGURE 2.—Predictions of χ activity decay models with respect to the activity of χ when buried in DNA sequence heterologies. Heterology length indicates the size of the DNA sequence heterology that χ must act across to stimulate recombination in interval I. Interval II is a χ-free control interval. χ activity = (number of interval I recombinants)/(number of interval II recombinants).

λ lytic crosses with free replication: Crosses were performed with seven infecting particles of each phage genotype (MOI = 7). Cells were grown at 34° with shaking in PC broth (Tryptone broth + 0.2% maltose + 10 μg/ml B1 + 5 mM $MgSO_4$) to 1×10^8/ml, harvested by centrifugation and resuspended in TM (10 mM Tris pH 7.4, 10 mM $MgSO_4$) to 1×10^8/ml as determined in a Petroff-Hausser counting chamber. Cells and phage were mixed in a total volume of 1 ml (volume

TABLE 1

λ genetic elements

Element	Properties
Asus32	Amber mutation in terminase gene *A*
χA	Chi mutation left of *b* region
Δ*b2*	*b* region deletion with no phenotype
Δ*b221*	Deletion of *b* region and *int*
Δ*b527*	*b* region deletion with no phenotype
Δ*b538*	Deletion of *b* region and *int*
Δ*b1453*	Deletion of *int, red* and part of *gam*
χ76	Chi mutation inseparable from *b1453*
bio1	Deletion of *int, red* and part of *gam* substituted by *E. coli* DNA from the *bio* operon
χ^{bio}	Natural chi site within the *E. coli bio* operon
int4	Unconditional *int* mutation
red3	Unconditional *red* mutation
gam210	Amber mutation in RecBCD inhibitor gene *gam*
cI26	Unconditional mutation in repressor gene *cI*
cI857	Temperature sensitive mutation in repressor gene *cI*
Rsus5	Amber mutation in lysozyme gene *R*

TABLE 2

Location of λ DNA deletion breakpoints on the λ physical map

Deletion allele	Left breakpoint[a]	Right breakpoint[a]
b221	19646	30445
b538	20809	29093
b2	21738	27731
b1453	27731	32915
bio1[b]	27731	33012
b527	23705	27731

[a] Deletion breakpoints (from DANIELS *et al.* 1983) refer to the DNA bp assignments of the left and right borders of the deletions on the λ physical map.

[b] The *bio1* allele deletes λ DNA at the indicated breakpoints and replaces it with *E. coli* DNA spanning from *attB* into the *bio* operon. This insertion introduces a χ site 3147 bp from the leftmost border of the deletion.

difference made up with TM), and phage adsorption proceeded at 37° with gentle aeration. After 10 min, 4 ml Cross broth (Tryptone broth + 0.3% glucose + 10 μg/ml B1 + 2 mM $MgSO_4$ + 75 μM $CaCl_2$ + 4 μM $FeCl_3$) was added. In crosses with *Eco*K-modified phage, cells were harvested by low speed centrifugation, the supernatants were removed and titered for unadsorbed phage, and the pellets were resuspended in 5 ml 37° Cross broth and diluted 40-fold in the same. In crosses with unmodified phage, unadsorbed phage were killed by restriction in the plating hosts. Phage adsorption exceeded 99.9%, and the two protocols gave essentially identical results. The cell suspensions were returned to the 37° water bath and vigorously aerated. After 75 min, 0.1 ml of egg white lysozyme (10 mg/ml) and 0.5 ml of fresh $CHCl_3$ were added, and the lysates were gently aerated for 15 min. Lysates were cleared of cell debris and residual $CHCl_3$ by centrifugation, transferred to detergent-free glass serum tubes and stored at 4°. Lysates were titered on fresh BBL Trypticase agar plates within 72 hr of completing the crosses. A^+ R^+ recombinant titers were determined by plating on *E. coli* strain AC417 (Su^- *recD1014*). Total phage titers were determined by plating on strain FS1576 (Su^+ *recD1009*). The *recD* alleles in AC417 and FS1576 permit λ *red gam* to form large plaques without regard to the χ genotype of the phage.

λ lytic crosses without DNA replication: FS2944 and FS3829 were grown in PC broth at 26° to 1 × 10^8/ml, harvested by centrifugation, resuspended in TM, starved for 20 min at 26° and shifted to 42.5° for 10 min to inactivate *dnaB*^{ts}*22* protein. Next, 5 × 10^7 cells and prewarmed phage were mixed in a total volume of 1 ml and incubated for 15 min with gentle aeration. The relative multiplicities in these crosses are indicated in the legends to Figures 5 and 6. After adsorption, 4 ml of 42.5° Cross broth was added, and the cultures were vigorously aerated. After 75 min, infected cells were harvested by centrifugation, and the supernatants were removed and titered for unadsorbed phage. Phage adsorption exceeded 90%. The cell pellets were resuspended in 4 ml Cross broth, 1 ml $CHCl_3$ and 0.2 ml lysozyme (10 mg/ml) and shaken at 34° for 30 min to lyse the cells. Lysates were harvested and treated as above. To verify that λ was not replicating, ^{13}C ^{15}N-labeled phage were included in parallel mock crosses and spun in cesium formate density gradients (MYERS *et al.* 1995). In each trial, a single density species of heavy phage was recovered, indicating that DNA replication was fully inhibited by the *dnaB*^{ts}*22* allele at this temperature.

Determination of χ activity: Dilutions of cross lysates were plated on AC417 to select for A^+ R^+ recombinants at 30 plaques per plate to minimize interplaque contamination. Individual plaques (separated by >3 mm from neighboring plaques) were harvested with glass straws into 1 ml TM buffer containing a few drops of $CHCl_3$ to kill AC417. The "pickates" were stored at room temperature until the $CHCl_3$ evaporated (~24 hr), at which time AC417 could no longer be detected. Portions of the pickates were spotted sequentially on AFT196 (Su^+ *recA56*) and V227 [Su^+ *recC1001* (P2)] to determine the *gam* allele present. The *gam210* mutation creates a nonsense codon that is suppressed in Su^+ strains. λ *red3 gam210* plates on AFT196 but not on V227 because inhibition of RecBCD enzyme by Gam permits λ *red3* to form packageable DNA concatemers in the absence of recombination but does not permit λ *red3* to overcome the inhibitory effect of the P2 *old* gene product. All of the other *gam* alleles used in this study eliminate the *red* and *gam* genes by deletion. λ deleted for *red* and *gam* plates on V227, but not on AFT196, because exo V activity destroys linear λ concatemers. This plating scheme allowed for unambiguous identification of the *gam* allele present in each pickate. Occasionally (<5 %), a pickate plated on both hosts. These probably arose from interplaque contamination during picking and were discarded from the analysis.

The *gam* genotype of a pickate was used to determine the position of the crossover that created the A^+ R^+ recombinant. Crossovers that arose to the left of the deletion are said to be in the "χ-stimulated" interval (interval I in Figures 3, 5 and 6). Crossovers that arose to the right of the deletion are said to be in the "control" interval (interval II in Figures 3, 5 and 6). In this paper, "χ activity" is defined as (number of interval I recombinants)/(number of interval II recombinants).

The deletions create heterology borders that both define the genetic intervals examined and limit homology to the left of χ. To make χ activities comparable between crosses, deletions were selected to minimize variation in the size of the homologous intervals; only the interstitial heterology was appreciably varied (Table 2). χ sites were embedded in the interstitial heterologies, *i.e.*, across from deletions (Figures 3, 5 and 6). "Heterology length" refers to the distance from χ to the leftmost border of the interstitial heterology defined by the opposing deletion.

RESULTS

Experimental design: Relative recombination rates in two intervals were monitored. χ stimulates recombination primarily to one side of itself, "leftward" on the standard λ map. To assess the effect of DNA sequence heterology on χ activity in each cross, the number of recombinants arising from exchange in an interval to the left of χ (interval I, the χ-influenced interval) was compared to the number of recombinants arising from exchange in an interval to the right of χ (interval II, the χ-free control interval). This χ activity was plotted as a function of increasing length of the DNA sequence heterology across which χ must act to stimulate exchange in interval I.

χ activity decreases with increased heterology length in λ lytic crosses with free replication: Heterology lengths were varied as illustrated in Figure 3. The results are summarized in Figure 4. The shortest heterology examined, 84 bp, had no effect on χ activity when compared to control crosses with χ present within interval I (*i.e.*, not acting across a DNA sequence heterology). In crosses with heterologies ranging in length from 84 to 3147 bp, χ activity decreased by a factor of 2.0 for

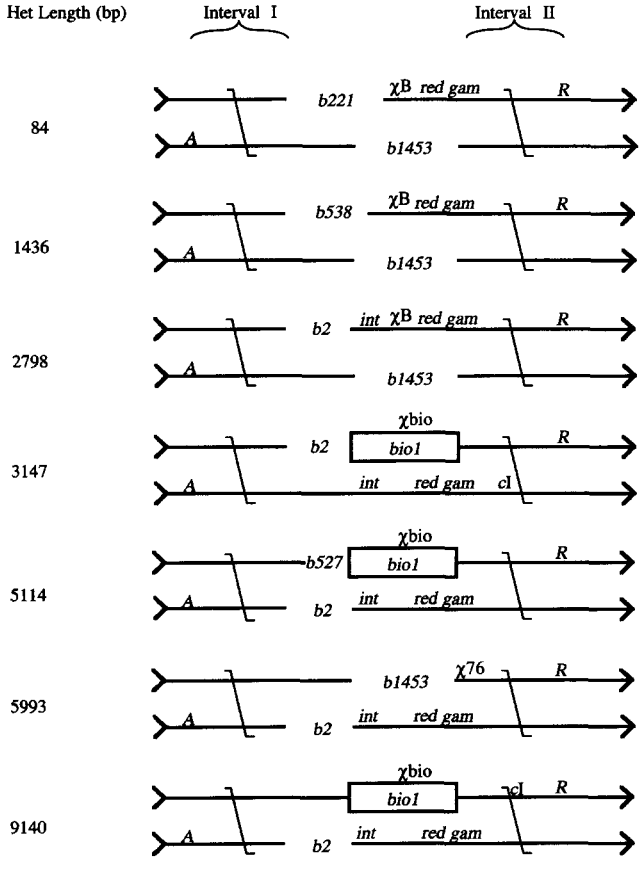

FIGURE 3.—Design of freely replicating λ crosses. Het length indicates the size of the DNA sequence heterology that χ must act across to stimulate recombination in interval I. Interval II is a χ-free control interval. The site χ76 is inseparable by recombination from the deletion *b1453*.

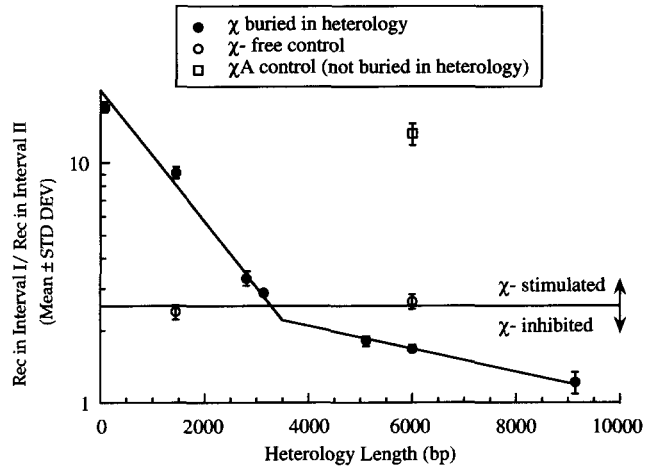

FIGURE 4.—Decay of χ activity in freely replicating crosses conducted in *rec*$^+$ cells. A$^+$ R$^+$ recombinants were selected, and individual plaques were isolated and tested to determine whether the crossover occurred in interval I (the χ-stimulated interval) or interval II (the χ-free control interval). For a given cross, interval I events were summed and divided by interval II events to assess the influence of χ in the cross. Each cross was conducted three times and the arithmetic means and standard deviations were determined. These values were plotted *vs.* the size of the heterology across which χ must act to stimulate recombination in interval I (Figure 3). (Recombination frequencies fluctuate to a greater extent than χ activity measurements, but the trends are consistent. Recombination frequencies, determined for freely replicating crosses, were as follows: Het 84, 5.4 ± 1.6%; Het 1436, 4.8 ± 2.2%; Het 2798, 4.2 ± 0.9%; Het 3147, 3.9 ± 1.8%; Het 5114, 1.7 ± 0.8%; Het 5993, 0.9 ± 0.3%; Het 9140, 0.9 ± 0.4%.) χ-free crosses employing derivatives of Het 1436 and Het 5993 phage and χA control crosses employing derivatives of Het 5993 phage were plotted to establish the limits of χ activity.

each 1.2 kb. This result is similar to previous reports on decay of χ activity with distance (LAM *et al.* 1974; ENNIS *et al.* 1987; CHENG and SMITH 1989), but with a slightly steeper slope. In crosses with heterologies between 5114 and 9140 bp, χ activity decayed by a factor of 2.0 for each 6.9 kb. However χ activity took on a different cast when the heterologies were >5114 bp; recombination in interval I appeared to be inhibited by χ to levels lower than in χ-free control crosses. The inhibition of χ activity by DNA sequence heterology could be due to either finite processivity of the χ-stimulated enzyme (RecBC) or to the luring of unwound DNA into nonproductive exchanges with chromosomes of identical genotype.

Heterology-induced decay of χ activity is caused by nonproductive exchanges: To determine if χ activity decreases with physical distance or genetic distance (*i.e.*, as a function of DNA length or *homologous* DNA length), we varied the relative multiplicity of homologous and heterologous targets for χ-activated exchange. DNA replication was inhibited to maintain a fixed ratio of homologous and heterologous phage in the crosses diagrammed in Figures 5 and 6. In Figure 5, the crosses were conducted at MOIs of 10 χ$^+$ to one χ-free phage and one χ$^+$ to 10 χ-free phage.

In crosses in which the χ-containing phage was in relative excess, χ activity declined sharply when the heterology length was increased from 84 to 2798 bp and then less sharply to a heterology of 9140 bp. In contrast, the χ activity in crosses in which the χ-free phage was in excess was barely inhibited by increasing the heterology length. These results indicate that the decrease in χ activity caused by DNA sequence heterology is due to matings of no genetic consequence (*i.e.*, exchanges between phage with identical genotypes inhibits χ activity in these crosses).

Varying the relative MOIs of the χ-containing and the χ-free phage in the above experiment alters not only the opportunity to recombine close to χ but also the number of available χ sites in the cell. In light of evidence that χ can act in *trans* to stimulate recombination (KÖPPEN *et al.* 1995; MYERS *et al.* 1995), we devised an additional test that did not vary the number of χ sites involved in the crosses.

To further test the model that DNA sequence heterology at χ inclines DNA unwound by RecBC into nonproductive exchanges with chromosomes of identical genotype, two types of crosses were conducted. In each cross, the multiplicity of the χ-containing genotype was fixed at one phage per cell. "Distracted" crosses were tripa-

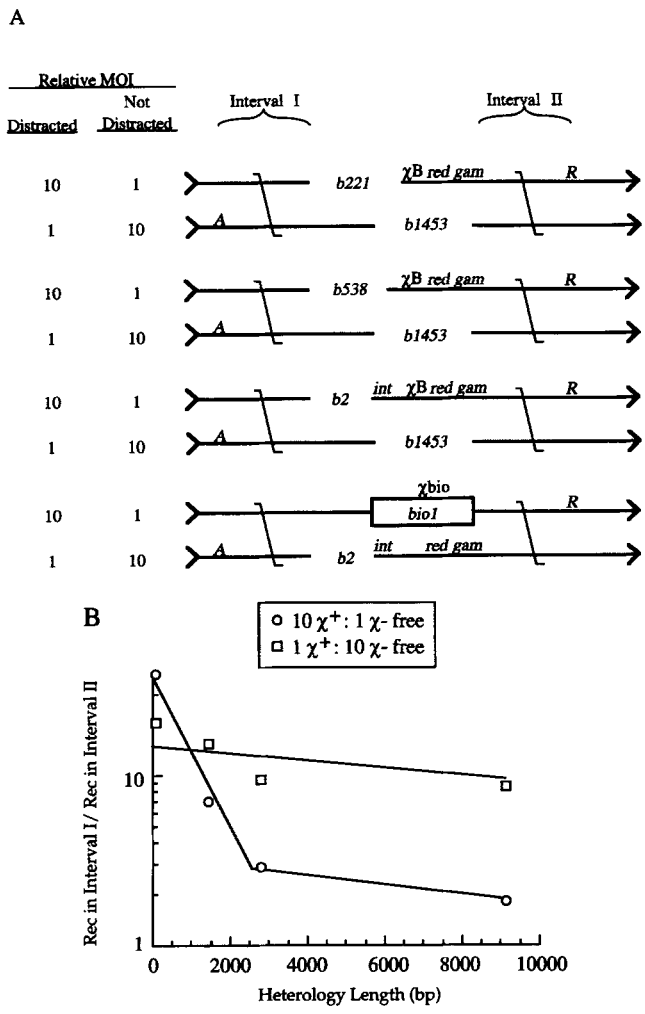

FIGURE 5.—χ activity declines due to nonproductive exchanges. (A) Design of crosses without DNA replication. λ were crossed in the absence of DNA replication to control in individual cells the numbers of phage with homology right up to χ and phage that are heterologous in the vicinity of χ. The relative MOI of χ^+ and χ-free phage were varied as indicated. (B) Phage were crossed in *rec*$^+$ *dnaBts E. coli* cells at 42.5° to inhibit DNA replication. Crosses employed 84-, 1436-, 2798-, and 9140-bp DNA sequence heterologies. The results are plotted as in Figure 4.

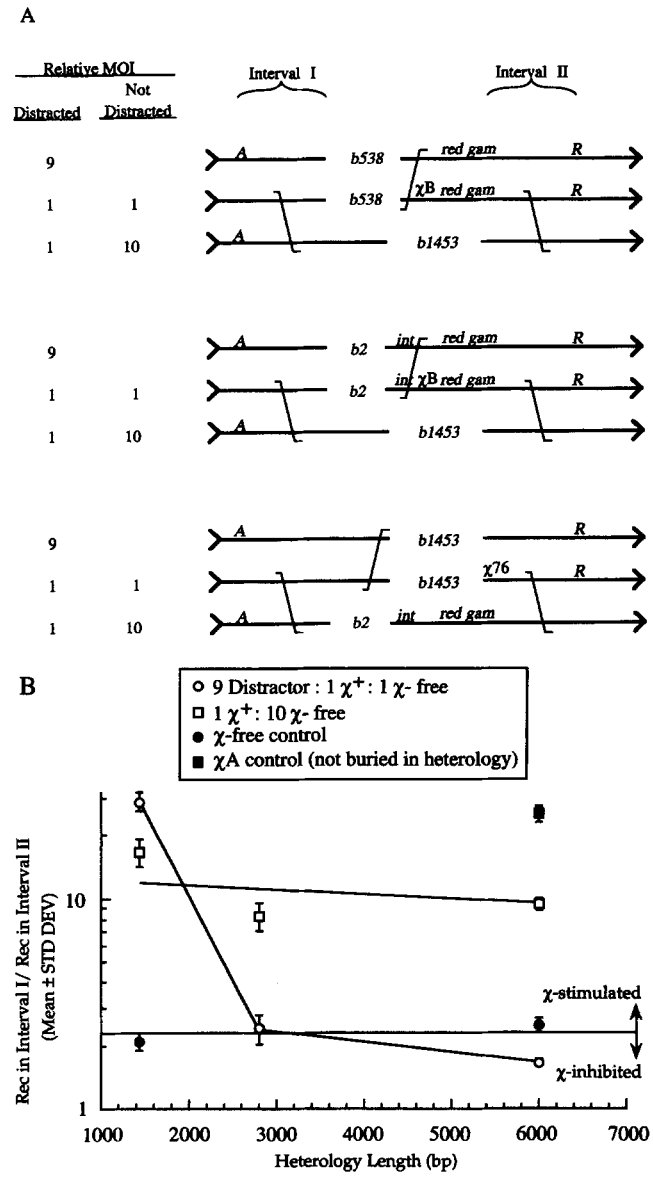

FIGURE 6.—χ activity declines only when homologous distractor phage are present. (A) Design of Distracted and Undistracted crosses. λ were crossed in the absence of DNA replication as in Figure 5. Distracted crosses were triparental with one χ+, one χ-free and nine distractor phage added per cell. Most opportunities to enjoy χ-stimulated exchange will be with phage that cannot contribute to the formation of A^+ R^+ recombinants. Undistracted crosses were biparental with one χ^+ and 10 χ-free phage added per cell. Most opportunities to catalyze χ-stimulated exchange will be with phage that can contribute to the formation of A^+ R^+ recombinants. (B) Phage were crossed in *rec*$^+$ *dnaBts E. coli* cells at 42.5° to inhibit DNA replication. Crosses employed 1436-, 2798-, and 5993-bp DNA sequence heterologies. χ-free crosses employing derivatives of Het 1436 and Het 5993 phage and χA control crosses employing derivatives of Het 5993 phage were plotted to establish the limits of χ activity. Each cross was conducted three times and the arithmetic means and standard deviations were determined. These values are plotted as in Figure 4.

rental, with one χ-containing phage, nine doubly marked "Distractor" phage and one heterologous phage. Distractor phage were doubly marked with mutant alleles to eliminate contributions of the distractor phage to the wild-type recombinant titer. Distractor phage and the χ-containing phage are homologous in the vicinity of χ. In contrast, the heterologous phage is heterologous at χ and shares sequence homology only distant from χ. "Undistracted" crosses were biparental, with one χ-containing phage and 10 heterologous phage.

Heterology lengths were varied as illustrated (Figure 6A), and the results are summarized in Figure 6B. In Distracted crosses, χ activity declined sharply when the heterology length was increased from 1436 to 2798 bp and then less sharply to a heterology of 5993 bp. In contrast, the χ activity in Undistracted crosses was not inhibited by increasing the heterology length. (The observed small rate of decrease in χ activity in the Undistracted crosses is consistent with the Poisson probability

of cells being infected by more than one χ-containing phage.) The results demonstrate that the reduction in χ activity with length of heterology is due to nonproductive exchanges between phage of the same genotype.

The role of ssDNA exonucleases in the χ activity gradient: It has been proposed that the function of χ is to convert RecBCD enzyme from a destructive exonuclease (exo V) to a recombinagenic DNA helicase (RecBC). ROSENBERG and HASTINGS (1991) noted that ssDNA exonucleases acting on DNA unwound by RecBC might alter the distribution of exchanges in the vicinity of χ. RINKEN *et al.* (1992) provided evidence that dsDNA unwound by the RecBC helicase is degraded by ssDNA exonucleases encoded by the *sbcB* and *recJ* genes. To assess the possible impact of exonucleases on the decay of χ activity, heterology crosses were repeated as in Figure 3 in a *sbcB recJ* double mutant background. The results are summarized in Figure 7A.

As in *rec*$^+$ cells, the 84-bp heterology did not diminish χ activity. However, the 1436-bp heterology caused a pronounced decrease in χ activity, and the 2793-bp heterology reduced χ activity to a level below that of χ-free control crosses. In crosses of phage with heterologies of 84 and 1436 bp, χ activity decreased by a factor of 2.0 for each 550 bp, a twofold increase in the decay rate of χ activity as a function of heterology length in *sbcB recJ* cells when compared to *rec*$^+$ *E. coli*. These results support the proposition that ssDNA exonucleases encoded by the *sbcB* and/or *recJ* genes act on the products of χ-activated RecBCD.

To determine the relative contributions of the ssDNA exonucleases encoded by the *sbcB* and/or *recJ* genes to χ-activity decay, heterology crosses were repeated in isogenic *rec*$^+$, *sbcB*, *recJ*, *sbcB recJ* cells. The results are summarized in Figure 7B. We conclude that the *sbcB*-encoded exonuclease I assists χ in acting across a DNA sequence heterology, whereas the *recJ*-encoded exonuclease gives no evidence of being involved in the gradient of χ activity.

Our crosses conducted in *sbcB* cells reveal a role for exonuclease I, a 3′ → 5′ ssDNA exonuclease, in promoting χ activity distant from χ. Previous *in vivo* studies (SIMMON and LEDERBERG 1972; RINKEN *et al.* 1992) indicated that this exonuclease degrades DNA unwound by RecBC helicase activity. There are two ways, not mutually exclusive, of thinking about the role of exonuclease I in helping χ to act across a heterology. (1) The position of exchange may be dictated by the position of the 3′ end, and exo I translates that end across the heterology. If that is so, we must enlist another nuclease (exonuclease VII?) that can translate ends across heterology in *sbcB* mutant cells. (2) The half-life of DNA unwound by RecBC opposite the heterology may be greater in exonuclease I-deficient cells. This increased longevity might increase the chance that the DNA will be decoyed into nonproductive exchange, thus decreasing the chance that it will be competent to recombine beyond the end of the heterology.

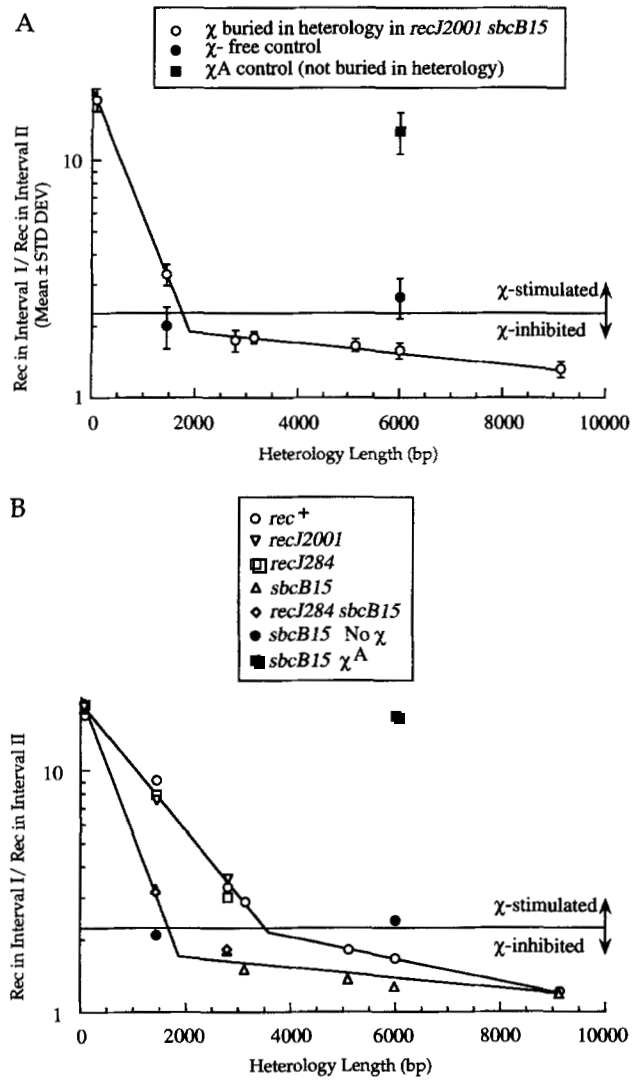

FIGURE 7.—The decay of χ activity is modulated by SbcB protein (exonuclease I). Crosses were conducted and plotted as in Figures 3 and 4. (A) Phage were crossed in *sbcB15 recJ2001*::Tn*10dKn* cells Each cross was conducted three times and the arithmetic means and standard deviations were determined. (B) Phage were crossed in *rec*+, *sbcB15*, *recJ284*::Tn*10*, *recJ2001*::Tn*10dKn*, and *sbcB15 recJ284*::Tn*10* cells. χ-free crosses employing derivatives of Het 1436 and Het 5993 phage and χA control crosses employing derivatives of Het 5993 phage were performed in *sbcB15* cells.

To determine if the increased inhibition of χ activity by DNA sequence heterology in *sbcB15* mutant cells is due to failure to expose homology at the 3′ end of DNA unwound by RecBC or due to increased opportunities for the unwound heterologous DNA to be lured into nonproductive exchange, we varied the relative multiplicity of homologous and heterologous targets for χ-activated exchange. In crosses in which the χ-containing phage was in relative excess, χ activity declined sharply when the heterology length was increased from 84 to 2798 bp and then less sharply to a heterology of 9140 bp. In contrast, the χ activity in crosses in which the χ-free phage was in excess was scarcely inhibited by increasing the heterology length. These results (Figure

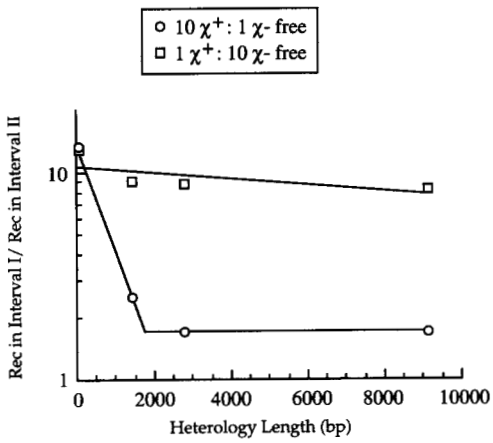

FIGURE 8.—The decay of χ activity in *sbcB15* cells is due to nonproductive exchanges. Crosses were conducted as in Figure 5 except in *sbcB15 dnaB*^{ts} cells.

8) indicate that the decrease in χ activity caused by DNA sequence heterology in *sbcB15* mutant cells, as in *rec*$^{+}$ cells, is due to exchanges of no genetic consequence.

DISCUSSION

Our crosses demonstrate that χ activity decreases not as a function of physical distance from χ, but rather with genetic distance. When χ is opposite a long heterology, nonproductive exchanges preempt productive exchanges, leading not only to a decrease in χ activity, but to an inhibition of χ activity when nonproductive exchanges approach saturation.

Our results also suggest that a given χ-activated RecBC molecule catalyzes but one recombination event (or coupled pair of events) (STAHL *et al.* 1990) when acting on a given chromosome. This single act of exchange can be distant from χ. In the Undistracted crosses, χ76-activated RecBCD travelled 5993 bp and retained the ability to catalyze exchange. The ability of χ-activated RecBCD to act across a 6-kb heterology suggests that χ sites do not act directly in recombination, but rather modify the activity of RecBCD enzyme. The ability of χ to act in *trans* (KÖPPEN *et al.* 1995; MYERS *et al.* 1995) has previously established this view.

In crosses between phage that are homologous in the vicinity of χ, χ activity decreases as distance from χ increases. In light of our results with heterologous crosses, we conclude that the decay of χ activity reflects the high probability of exchange in the vicinity of χ, with consequent loss of the ability to enjoy downstream exchanges.

χ activity persists unabated over distances greater than had been previously recognized. Apparently, RecBC enzyme unwinds as much DNA as is required to catalyze exchange. The limits of DNA unwinding *in vivo* are unknown for RecBC, but *in vitro* RecBC enzyme unwinds, on the average, 2.8 kb of DNA in a single pass [our estimate from the data of KORANGY and JULIN (1993)].

What is the origin of the upward curvature of the dependence of χ activity on heterology length (Figures 4–8)? Our demonstration that decay of χ activity to the left of χ depends on the availability of homology suggests an explanation. Some of the infecting χ-containing phage may find themselves in circumstances where there are few or no homologues available to distract the exchange reaction. The random adsorbtion of phage to cells plus the failure of some infecting chromosomes to replicate (MESELSON and WEIGLE 1973) could contribute to such a circumstance. For these phage χ activity will be relatively distance-independent; at long distances these phage will contribute disproportionately to the enumerated recombinants. A second contribution to the concavity is likely to be from the rather frequent failure of RecBCD to recognize an encountered χ (STAHL *et al.* 1990). On those occasions χ activity will mimic the χ-free control.

Our crosses conducted in *sbcB15* cells revealed a role for exonuclease I, a 3′ → 5′ ssDNA exonuclease, in promoting χ activity distant from χ. PHILLIPS *et al.* (1988) previously determined that the *sbcB15* mutation greatly reduces (but does not eliminate) exonuclease I activity. Our results suggest that the half-life of DNA unwound by RecBC opposite the heterology is greater in exonuclease I-deficient cells and that this increased longevity increases the chance that the DNA will be decoyed into nonproductive exchange, thus decreasing the chance that it will be competent to recombine beyond the end of the heterology. Since the *sbcB15* mutant protein retains some function(s) (PHILLIPS *et al.* 1988), we cannot rule out the possibility that the enhanced decay in χ activity is due to some unknown property of the mutant employed.

The RecJ nuclease also degrades DNA unwound by RecBC helicase activity (RINKEN *et al.* 1992). However, we did not observe that RecJ nuclease activity altered the distribution of χ-mediated exchanges. This result is consistent with two possibilities: (1) the 5′-tipped ssDNA chain may not participate in χ-mediated splices and (2) the 5′-tipped ssDNA chain may participate only in subterminal χ-mediated events, which do not require degradation beyond the heterology (see MYERS and STAHL 1994). HAGEMANN and ROSENBERG (1991) determined that 5′-tipped ssDNA chains participated in a significant majority of χ-mediated patches.

The early steps of recombination in the RecBCD pathway appear to be linearization of dsDNA by accident or design, degradation of the linearized DNA duplex by the exo V activity of RecBCD enzyme, χ recognition by RecBCD enzyme, attenuation of exo V by loss or inactivation of the RecD subunit, continued DNA unwinding by RecBC enzyme, (limited?) degradation of ssDNA tails by exonuclease I (and other exonucleases?), assembly of a RecA filament on ssDNA, and synapsis with an intact portion of a homologous DNA duplex. Subsequent steps presumably involve the generation of intermediate(s) whose resolution may be effected by

DNA synthesis and/or by the endonucleolytic action of "resolvases".

KAISER (1955) analyzed recombination in phage λ within the pairwise mating scheme of VISCONTI and DELBRÜCK (1953). In this scheme, two phage chromosomes pair along their length and, while doing so, may enjoy several exchanges (a mating). Phage may enter into successive pair-wise matings. Our results, relevant only to the RecBCD pathway of recombination, show that the pairwise commitment can be compromised when gratification is excessively delayed by heterology. With somewhat similar experiments, the Visconti-Delbrück theory has previously been shown to be inapplicable to phage T4 (DRAKE 1967).

We appreciate the support and comments, both scientific and aesthetic, of the members of the laboratory. SUSAN ROSENBERG (Edmonton) reminded us that *sbcB15* is not a null allele. R.S.M. was supported in part by National Institutes of Health National Research Service Award 5F32GM-14196. This work was supported by National Science Foundation grant MCB-9402695 and by U.S. Public Health Service grant GM-33677. F.W.S. is American Cancer Society Research Professor of Molecular Genetics.

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Communicating editor: P. L. FOSTER