Distribution of Chi-Stimulated Recombinational Exchanges and Heteroduplex Endpoints in Phage Lambda

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Manuscript received December 27, 1988
Accepted for publication May 15, 1989

ABSTRACT

The recombination hotspot Chi, 5′ G-C-T-G-G-T-G-G 3′, stimulates the RecBCD recombination pathway of *Escherichia coli*. We have determined, with precision greater than previously reported, the distribution of Chi-stimulated exchanges around a Chi site in phage λ. Crosses of λ phages with single base-pair mutations surrounding a Chi site were conducted in and analyzed on mismatch correction-impaired hosts to preserve heteroduplex mismatches for analysis. Among phages recombinant for flanking markers, Chi stimulated exchanges most intensely in the intervals immediately adjacent to the Chi site, both to its right and to its left. Stimulation fell off abruptly to the right but gradually to the left (with respect to the orientation of the Chi sequence written above). We have also determined that Chi stimulated the formation of heteroduplex DNA, which frequently had one endpoint to the right of Chi and the other endpoint to the left. These data support a model of Chi-stimulated recombination in which RecBCD enzyme cuts DNA immediately to the right of Chi and unwinds DNA to the left of Chi; segments of unwound single-stranded DNA are sometimes, but not always, degraded before synapsis with homologous DNA.

SPECIAL sites in both eukaryotes and prokaryotes affect the frequency and distribution of recombination events in their vicinity (STAHL 1979; SMITH 1988b). Chi, 5′ G-C-T-G-G-T-G-G 3′ (SMITH *et al.* 1981a), enhances break-join recombination (LAM *et al.* 1974) mediated by the primary pathway of recombination of *Escherichia coli,* the RecBCD pathway (STAHL and STAHL 1977) (reviewed by STAHL 1979 and SMITH 1987). Models of Chi-stimulated recombination incorporating known genetic and biochemical properties of Chi and the RecBCD pathway enzymes have been proposed (STAHL 1979; Smith *et al.* 1981b). In this paper, we test predictions of one of these models by analyzing recombinants from phage λ Red$^-$ Gam$^-$ crosses conducted in and analyzed on mismatch correction-impaired hosts.

Two known components of the RecBCD pathway (CLARK 1973; SMITH 1988a), RecA protein and RecBCD enzyme, have been studied *in vitro* in detail (for reviews, see COX and LEHMAN 1987; TAYLOR 1988). RecA protein promotes interactions between homologous DNA molecules, including strand transfer between double-stranded and single-stranded DNA (SHIBATA *et al.* 1979). RecBCD enzyme makes single strand endonucleolytic cleavages four to six nucleotides to the right of the Chi octamer as the enzyme unwinds the DNA from right to left (with respect to Chi as written above; PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985). The frequency of DNA strand cleavage strongly correlates with the recombinational hotspot activity of mutant forms of RecBCD enzyme (PONTICELLI *et al.* 1985) and of mutant forms of Chi (CHENG and SMITH 1987). This correlation indicates that the cleavage about five nucleotides to the right of Chi is essential for hotspot activity of Chi.

Does the cleavage to the right of Chi correspond to a position of Chi-stimulated exchange? Previous studies have not determined the distribution of Chi-stimulated exchange with sufficient precision to answer this question. Analysis in density gradients of the progeny from crosses of isotopically labeled parental λ phage (*e.g.*, LAM *et al.* 1974; STAHL, CRASEMANN and STAHL 1975; STAHL *et al.* 1980) has a resolution of several thousand base-pairs (kb) and hence cannot provide the resolution required to answer this question. Crosses employing a large heterology at Chi (*e.g.*, STAHL *et al.* 1980) can detect exchange only outside the heterology, since there is no evidence that Chi stimulates nonhomologous exchange. There has been only one previous report that Chi could stimulate exchange to its right (relative to the conventional λ map); in that report (STAHL and STAHL 1975) χ^+C opposite the large heterology imm^{21} stimulated exchange in the interval imm^{21}-*R* to the right of Chi. However, this rightward stimulation has not been reproducible (M. M. STAHL and F. W. STAHL, personal communication). Several studies (*e.g.*, STAHL

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and STAHL 1975; STAHL *et al.* 1980; CHENG and SMITH 1984) have shown that Chi stimulation is greater to its left than to its right, but the absolute amount of stimulation to the right is unknown, since stimulations to the left and right were measured only relative to each other. Two experimental features are essential to determine if Chi can stimulate exchange immediately to its right or left: use of Chi as a marker and homology at Chi. In one report (CHENG and SMITH 1984) using these features there were more Chi-stimulated exchanges to the left of Chi than to its right, but again only relative stimulations were measured. In addition, the absence of additional nearby markers in that report precluded determining how close to Chi (or far from it) these exchanges occurred. To determine the locations of Chi-stimulated exchanges, we have conducted λ Red$^-$ Gam$^-$ crosses with Chi and additional, nearby single base-pair mutations as markers.

In one model of Chi-stimulated recombination (SMITH *et al.* 1981b, 1984), RecBCD enzyme cuts one DNA strand to the right of Chi as it unwinds the DNA from right to left (Figure 1). Continued unwinding produces a single-stranded DNA tail extending leftward from Chi, which invades a homologous DNA duplex in a RecA protein-promoted reaction. This model predicts that all unreplicated Chi-stimulated recombinants have hybrid DNA at Chi (Figure 1, step I). After replication, half of the Chi-stimulated recombinants exchanged for flanking markers (step I, right) are predicted to have their point of genetic exchange to the left of Chi, and the other half immediately to the right of Chi. If the single-stranded DNA tail extending leftward from Chi ("Chi tail") were degraded more than about 10 nucleotides from its 3′ end, the recombinants would not be hybrid at Chi and both genetic exchange points would be to the left of Chi. In this paper we test these predictions and estimate the frequency of the hypothesized degradation of the Chi tail from the distribution of exchanges among phages recombinant for flanking markers and from the deduced endpoints of heteroduplex DNA in the Chi-stimulated recombinants.

Genetic heterozygosity signaling the presence of hybrid DNA can be abolished by mismatch correction (WILDENBERG and MESELSON 1975; Figure 2). Therefore, mismatch correction-impaired hosts, such as *mutL* strains (MODRICH 1987; RAPOSA and FOX 1987), are more likely to yield recombinants reflecting the initial break-join exchanges than are crosses permissive for mismatch correction. For example, HUISMAN and FOX (1986) found that the frequency of lambda O^+P^+ recombinants heteroduplex at *cI* is 10–20-fold higher when the cross lysate is plated on *mutL218*::Tn*10*, rather than *mutL*$^+$, indicator bacteria;

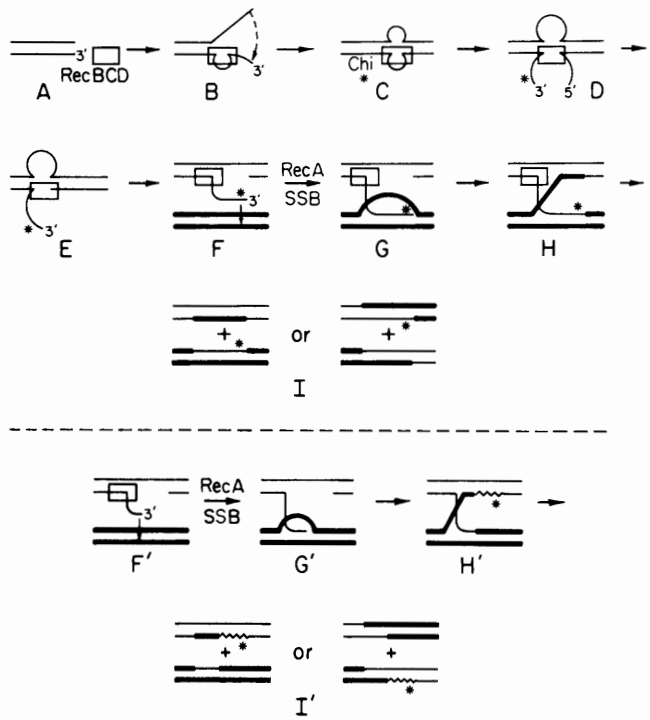

FIGURE 1.—Model for RecBCD pathway recombination. One parental chromosome is represented by a pair of light lines, and the other (which may be circular, but is drawn as a linear molecule for simplicity) by a pair of heavy lines. RecBCD enzyme (box) attaches to a duplex end (A), unwinds the DNA to form a loop plus short tail (B) on the strand with a 3′-end (BRAEDT and SMITH 1989). The ends of the loop-tail anneal to form a twin-loop (C) (TAYLOR and SMITH 1980). Upon encountering a properly oriented Chi sequence, RecBCD enzyme cuts the strand containing 5′ G-C-T-G-G-T-G-G 3′ (Chi, denoted by * in steps D to I) to produce a 3′ ended "Chi tail" (F) (PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985). RecA and SSB proteins promote synapsis between the Chi tail and the homologous parent to form a D-loop (G) (COX and LEHMAN 1987). Cleavage of the D-loop, perhaps by RecBCD enzyme, followed by synapsis of the displaced strand with the gap in the first parental DNA forms a Holliday junction (H), whose migration may be driven by RecBCD enzyme. The Holliday junction is resolved by cutting, perhaps by RecBCD enzyme, and ligation of homologous strands to produce recombinants with parental or recombinant configurations of flanking markers (left and right of I, respectively). Panels below the dashed line diagram the consequences of degradation of the 3′ ended "Chi tail" (F) to produce a shorter tail lacking the Chi sequence (*)(F′). Synapsis to form a D-loop (G′) and formation (H′) and resolution (I′) of the Holliday junction proceed as above, except that repair synthesis (〰) replaces the nucleotides lost during degradation of the "Chi tail" (F to F′). Consequently, hybrid DNA does not cover the Chi site, and the points of Chi-stimulated recombinational exchanges are displaced to the left. The repair synthesis drawn here is primed by a 3′ end after formation of the Holliday junction (H′). Alternatively, repair synthesis could be primed by a 3′ end before formation of the Holliday junction (G′); enlargement and annealing of the D-loop to the gap would form hybrid DNA at Chi and yield genetic exchange to the right of Chi. The genetic consequences of these steps are discussed by SMITH *et al.* (1984). (Modified from SMITH *et al.* 1981b.)

mutL in the bacterial host for the lambda cross has an insignificant effect.

To detect heteroduplex-containing recombinants, we have used two clear plaque morphology markers:

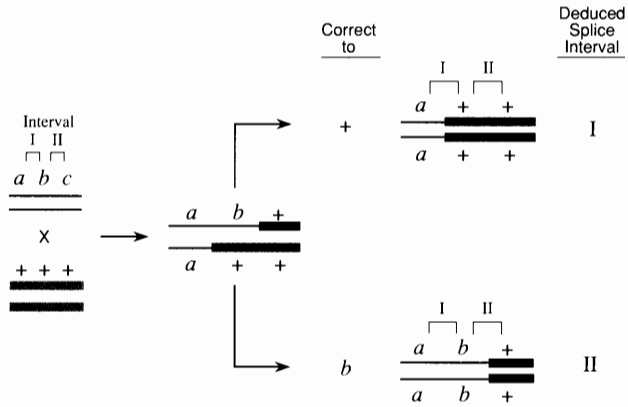

FIGURE 2.—Misscoring of splice points due to mismatch correction. A splice point, or point of genetic exchange, is the point where DNA from one parent is connected to DNA from the other parent. Starting with parents *a b c* and + + + (with intervals I and II defined by the *a-b* and *b-c* intervals, respectively), a recombinant heteroduplex at *b* is generated. Correction of the *b*/+ mismatch to "+/+" produces an *a* + + recombinant, suggesting that two single strand exchanges have occurred in interval I. Correction of the mismatch to "*b*/*b*" produces an *a b* + recombinant, suggesting that both exchanges have occurred in interval II. Either suggestion is incorrect; the recombinant had one exchange point in each interval.

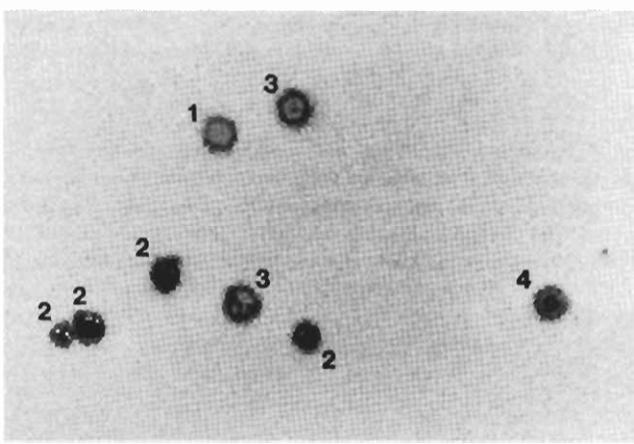

FIGURE 3.—Turbid, clear and mottled plaques. The photograph, about two times life size, is from a plating of the χ^+ cross diagrammed in Figure 6 on strain V351. To allow different plaque types to be photographed close together, the cross was plated at higher density than was done for cross analyses. Plaque types are as follows: 1, very turbid (*c*$^+$); 2, very clear (*cI*); 3, mottled (*cI*/*c*$^+$); 4, slightly turbid (*cII*).

cI60 (KAISER 1957) and χ^+C, which is a recombinational hotspot (STAHL, CRASEMANN and STAHL 1975) as well as a plaque morphology marker in *cII*. Thus, recombinants heteroduplex for either or both of these markers were detected as mottled plaques (Figure 3) on *mutL*::Tn*10* indicator bacteria. In addition, we screened recombinants for heteroduplex DNA at two additional markers, *susN7* and *susO29*, to the left and right of the *c* markers, respectively.

Recombination of λ Red$^-$ Gam$^-$ by the RecBCD pathway occurs at a low level in the absence of the canonical Chi sequence. To determine the contribution of that "background" to recombination occurring in the presence of Chi, a "χ^- cross" containing the inactive χ^-C209 sequence 5′ G-C-T-G-G-T-AG 3′ (SCHULTZ, SWINDLE and SMITH 1981; CHENG and SMITH 1984) in place of Chi, was done in parallel with each χ^+ cross.

TABLE 1

***Escherichia coli* strains used**

Strain	Genotype	Source or reference
V322	*mutL218*::Tn*10 supE argE*(am) *metB gyrA*(Nal^r) *rpoB*(Rif^R) del(*lac-pro*)	HUISMAN and FOX (1986) (strain D6432)
594	*lac-3350 galK2 galT22 rpsL179*	WEIGLE (1966)
V348	as 594 plus *mutL218*::Tn*10*	V322 × 594[a]
V228	*supE recC1001 argA his gal endA tonA*	D. W. SCHULTZ (strain D241)
V227	as V228 plus (P2)	Lysogen of V228
V351	as V228 plus *mutL218*::Tn*10* (P2)	V322 × V227
JC8679	*recB21 recC22 sbcA23 supE44 thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 proA2 his-4 argE3 rpsL31 tsx-33 mtl-1*	GILLEN (1974)
JC9387	*recB21 recC22 sbcB15 thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 proA2 his-4 argE3 rpsL31 tsx-33 mtl-1*	GILLEN (1974)
V350	as JC9387 plus *mutL218*::Tn*10*	V322 × JC9387
JM1	*recB21 sbcA20 supF his-318 leu*(am) *lac*(am?)	STAHL *et al.* (1980)

[a] *A* × *B*, P1 transduction where *A* is the donor and *B* is the recipient. Also see MATERIALS AND METHODS.

MATERIALS AND METHODS

Bacterial strains: Bacterial strains are listed in Table 1. The MutL phenotype of strains V348 and V351 was determined by their higher frequency of spontaneous mutation to spectinomycin-resistance (SIEGEL and IVERS 1975) relative to the parental strains 594 and V227, respectively.

Phage strains: Phage lambda strains are listed in Table 2. Strains were derived by lytic crosses between phage as described by SCHULTZ, SWINDLE and SMITH (1981) and from our collection.

Culture media: Supplemented tryptone broth (STB) contains 1% (all percents are w/v) Bacto-tryptone (Difco, Detroit, MI), 0.5% NaCl, 0.1% maltose, 0.5 μg/ml thiamine, and 2.5 mM $MgSO_4$. BBL agar is 1% Trypticase peptone (BBL, Cockeysville, MD), 1% Bacto-agar (Difco) and 0.5% NaCl. BBL top agar contains 0.65% Bacto-agar (Difco), 0.5% NaCl and 1.0% Trypticase peptone (BBL). BBL-YE agar is BBL agar containing 0.5% Bacto-yeast extract (Difco). SM is 0.5% NaCl, 1 mM $MgSO_4$, 20 mM Tris-HCl (pH 7.4) and 0.01% gelatin.

Lambda crosses: For each cross, bacteria were grown exponentially in STB at 37° to a density of about 3 x 10^8 colony-forming units (cfu) per ml. An aliquot of 0.2 ml of parental phage mixture in SM was added to 0.2 ml of bacterial culture to give a phage/cfu ratio of 5 for each parent. After 20 min stationary incubation at 37°, the mixture was diluted 100-fold in STB, incubated on a rotating drum at 37° for 2 hr, sterilized with a few drops of chloroform and stored at 4°. This lysate was diluted in SM, mixed with about 1 x 10^8 plating bacteria freshly grown in

TABLE 2

Phage lambda strains used

Strain	Genotype
1429	*b1453 χ⁺C151*
1503	*red-3 gam-210 cI60*
1556	*b1453 χ⁺C151 χ⁻C209*
1568	*b1453 χ⁺C151 susS7*
1569	*b1453 χ⁺C151 χ⁻C209 susS7*
1570	*b1453 cI60*
1581	*b1453*
1623	*red-3 gam-210 susN7 cI60*
1647	*b1453 susN7 cI60*
1650	*b1453 susO29*
1651	*b1453 χ⁺C151 susO29*
1652	*b1453 χ⁺C151 χ⁻C209 susO29*
1686	*red-3 gam-210 cI60 susO29*

Sources of the alleles are as follows: *b1453*, HENDERSON and WEIL (1975); *χ⁺C151*, STAHL, CRASEMANN and STAHL (1975); *susS7*, GOLDBERG and HOWE (1969); *χ⁻C209*, SCHULTZ, SWINDLE and SMITH (1981); *red-3*, SIGNER and WEIL (1968); *gam-210*, ZISSLER, SIGNER and SCHAEFER (1971a); *susN7* and *susO29*, CAMPBELL (1961); *cI60*, KAISER (1957).

The following data were used for calculation of physical distances between phage lambda markers in Figures 4, 5 and 6: The nucleotide positions of the *gam* gene and markers *susN7* and *susS7* are given by SANGER *et al.* (1982) and that of the *susO29* marker by RADICELLA, CLARK and FOX (1988). The *b1453* deletion ends within the *gam* gene (HENDERSON and WEILL (1975); for our calculations the right endpoint is assumed to be in the middle of the gene. *cI60* is in the right part of *cI* (SMITH 1975), and for our calculations is assumed to be 10% from the right end.

STB to about 5 x 10^8 cfu/ml, incubated at 37° for 20 min, and plated with 2.5 ml BBL top agar on 90-mm Petri plates containing about 40 ml BBL-YE agar per plate poured 1–2 days previously. Plates were incubated at 37° for 40–72 hr before scoring of plaque morphologies. The crosses in Figures 4, 5 and 6 were done on 3 separate days; aliquots of the same bacterial cultures and sets of plates were used for each χ^+ and χ^- pair.

The basis of the selection for recombinants for flanking markers is as follows: *gam-210* is suppressed by *supE* (ZISSLER, SIGNER and SCHAEFER 1971a), while the deletion *b1453* confers an unconditional Red⁻ Gam⁻ phenotype (HENDERSON and WEIL 1975). P2 lysogens support the growth of only Red⁻ Gam⁻ derivatives of λ (ZISSLER, SIGNER and SCHAEFER 1971b), and *susS7* is not suppressed by *supE* (GOLDBERG and HOWE 1969). Therefore, only *b1453* S^+ recombinants grow on the *supE* (P2) strain V351. Because the morphology of tiny plaques cannot be scored reliably, strain V351 also contains *recC1001*, which results in an elevated level of recombination compared to *rec*⁺, allowing χ^- Red⁻ Gam⁻ to form plaques as large as their χ^+ Red⁻ Gam⁻ counterparts (SCHULTZ, TAYLOR and SMITH 1983).

For the crosses in Figures 5 and 6, plaques were scored as Sus if they did not grow after stabbing with sterile toothpicks to lawns of the *sup*⁺ strain JC9387 but did grow on the *supE* strain JC8679; phage were scored as Sus⁺ if they grew after stabbing to both *sup*⁺ and *supE* strain lawns.

Initial scoring of plaque morphologies: Each plaque on the plates was identified by morphology and numbered; note was also taken of proximity to plaques less than 10 mm away (see below under *Detection of mixed plaques*). The categories of plaques scored were: very clear, slightly turbid, very turbid, mottled and indeterminate (see Figure 3 for examples of plaque morphologies). Indeterminate plaques included any which could not be readily scored by inspection, such as small plaques and plaques at plate edges. All indeterminate and mottled plaques were retested by poking with a fine platinum needle with a spherical end and streaking on lawns of the *supE recC1001* (P2) strain V351 on BBL-YE plates.

Detection of mixed plaques: To harvest the progeny from individual recombinant phage, agar plugs containing individual plaques were picked using sterile Pasteur pipettes into 1 ml of SM plus chloroform and vortexed ("pickates"). To determine the frequency of mixed plaques among recombinants with each morphology, phages from randomly chosen plaques of each morphology were tested by streaking on lawns of the *supE recC1001* (P2) strain V351 on BBL-YE plates. Among about 50 very clear plaques from different χ^+ and χ^- crosses (Figure 5) and 50 slightly turbid plaques from χ^- crosses, none were mixed. Mixed plaques were detected among slightly turbid plaques from the χ^+ cross and very turbid plaques from both χ^+ and χ^- crosses. Therefore, all mottled and turbid plaques, all slightly turbid plaques from χ^+ crosses and about 10% of the slightly turbid plaques from χ^- crosses were tested for being mixed. Individual mixed plaques occasionally contained minority populations as low as about 10%. Thus, a plaque was scored as mixed if the minority plaque type made up at least 10% of progeny phage and a plaque of the minority class was not present on the original plate less than about 1.5 mm away (closer plaques can give cross-contamination; see below). Pickates were restreaked when the proportions of plaque types were not obvious on first streaking. Plaques found to be mixed at *cI* or *cII* in the crosses of Figures 5 and 6 were also scored for Sus as described above.

To test for plaques mixed at *susN7* among very clear (*cI*) plaques from the Figure 5 crosses, the very clear plaques were first scored for Sus as described above. To distinguish between recombinants that were N^+ on both strands from those that were N^+/*susN7* heteroduplex (both score as Sus⁺ by the stab test), all Sus⁺ recombinant phage were streaked from the *supE* strain JC8679 stab plate onto lawns of JC8679 to obtain at least 8 isolated (step 2) plaques. Step 2 plaques were tested for Sus as described above. When all of the step 2 plaques were Sus⁺, the original *cI* recombinant was scored as N^+ on both strands. When one or more of the step 2 plaques were Sus, the original *cI* recombinant was scored as N^+/*susN7* heteroduplex.

To test for plaques mixed at *susO29* among very turbid (c^+) and slightly turbid (*cII*) plaques from the Figure 6 crosses, plaques were first scored for Sus and then streaked and stabbed as described above for scoring of N^+/*susN7* heteroduplexes.

To test how far phage can diffuse from their plaques into contiguous agar, about 50 restreakings were done at different distances from plaques in platings of different crosses. Phage were found to diffuse as far as about 1.5 mm from a plaque after 3 days' incubation. To minimize the chance of plaques being too close to each other, platings were done to yield about 40 or fewer recombinant plaques per plate.

Other controls: To test the relative efficiencies of plating of all recombinant types, plate stocks of phage with the genotypes of the predicted recombinants from each cross were plated on the *supE* strain JC8679 and on *supE recC1001* (P2) strain V351. For all recombinant genotypes, the relative efficiencies of plating on these two strains were within 15% of each other and not consistently higher or lower on either strain on five occasions for the crosses diagrammed in Figures 4 and 5 and on two occasions for the crosses diagrammed in Figure 6.

To test whether recombinants can arise following plating (for the Figure 4 crosses), two sets of controls were done.

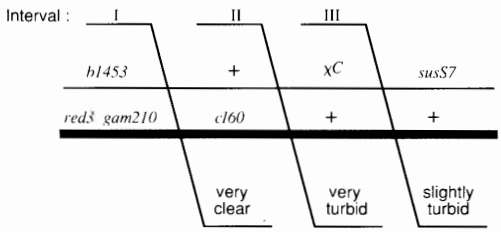

FIGURE 4.—Crosses to determine the distribution of Chi-stimulated exchange. The diagram represents two crosses done in parallel: in one, the upper parent (phage 1568) contains χ^+*C151* (Chi, 5′ G-C-T-G-G-T-G-G 3′) at χC, and in the other, the upper parent (phage 1569) contains the χ^-*C209* octamer (5′ G-C-T-G-G-T-A-G-3′) at χC (underlined bases are changes from wild type) (SCHULTZ, SWINDLE and SMITH 1981). The lower parent for both crosses is phage 1503. The slanted lines represent the three possible locations of exchange in the selected *b1453* S^+ recombinants (represented in Figure 1 by the upper right recombinant in step I). Crosses were done as described in MATERIALS AND METHODS. The basis of the selection, scoring for genetic exchange in the three intervals, and scoring for mottled (heteroduplex-containing) plaques are also described in MATERIALS AND METHODS. Distances between markers are not drawn to scale.

First, χ^+ and χ^- "cross lysates" were reconstructed using mixtures in SM of (1) parental phage alone, (2) parental phage plus one pure recombinant genotype phage at a time (50–100 recombinants per plate) and (3) parental phage plus all expected recombinant phage types (strains 1570, 1581, 1429 and 1556; 50–100 plaques per plate) on *supE recC1001* (P2) strain V351 in proportions expected in the cross lysates (from earlier platings for total and for recombinant phage). Reconstructions 1, 2 and 3 yielded no plaques, plaques of only the recombinant type added, and plaques of all types, respectively, in expected numbers. No mottled plaques were observed in any of these platings; 8 to 10 plaques of each type were streaked out and retained their original morphology.

The second set of controls, using the χ^+ cross of Figure 6, was designed to determine whether "rescue" of parental genomes (particularly the *susS7* parent, which can grow in nonsuppressing strains without cell lysis) can yield mixed plaques on the plating bacteria. From a typical plating for recombinants on *supE recC1001* (P2) strain V351, we picked five well-isolated, individual plaques of each morphological type: very clear, slightly turbid and very turbid. Platings of 0.05 ml of a 10^{-2} dilution of each pickate yielded about 500 plaques on the permissive *supF recB sbcA* strain JM1 (which would allow parents, as well as recombinants, to grow) on BBL-YE plates; all plaques were of the original rather than parental plaque type. Plating of 0.2 ml (undiluted) of five pickates from areas of bacterial lawn not containing visible plaques formed no plaques on JM1. Together, these results indicate that recombination between surviving parental phage and recombinants cannot account for mixed plaques.

To test for accurate scoring of *cI* and *cII* genotypes, 10 *cI* (very clear) and 8 *cII* (slightly turbid) recombinant plaques, and 18 *cI* and 6 *cII* plaques from streakings of mixed plaques from the Figure 4 χ^+ cross, were tested for complementation by spotting with known *cI* or *cII* phage (KAISER 1957). All complementation results were consistent with the initial scorings. No *cI cII* double mutants (expected to be *cI* by visual scoring) were observed.

RESULTS

Three sets of λ Red$^-$ Gam$^-$ crosses were analyzed to determine the distribution of Chi-stimulated exchange and to determine the frequency and extent of Chi-stimulated heteroduplex regions. The λ phages were Red$^-$, to eliminate λ's own homologous recombination system, and Gam$^-$ to eliminate λ's inhibitor of the RecBCD enzyme (for a review see SMITH 1983). The first set of crosses, containing the fewest markers, is the simplest; the second and third sets incorporate additional markers to the left and to the right of Chi, respectively. Each set consisted of a χ^+ and a χ^- cross conducted and analyzed concurrently (Figures 4, 5 and 6). The *b1453* S^+ recombinants from these crosses were analyzed to determine: (1) the overall distribution of exchanges (of which heteroduplex-containing recombinants comprise a subset) and (2) the structures and frequencies of heteroduplexes detected among those recombinants. (Recombinants parental for flanking markers were not examined.) The results of the three sets of crosses are compared at the end of the RESULTS (see Table 9).

The first set of crosses is represented in Figure 4, and the overall distribution of exchanges is presented in Table 3. The frequency of recombinants with exchanges in each interval was calculated from the total phage titers and the titers of recombinants with exchanges in each interval. For intervals I and II, to the left of Chi, the recombinant frequencies from the χ^+ cross were 5.9- and 5.5-fold higher, respectively, than those from the χ^- cross. For interval III, to the right of Chi, the corresponding χ^+/χ^- ratio was 0.73. These results show that Chi stimulated recombination strongly to its left but not detectably, by this measure, to its right.

To determine the frequency of exchange per unit physical distance, we calculated the percentage of all *b1453* S^+ recombinants with exchanges in each of the three intervals and divided these percentages by the percentage of the physical distance between *b1453* and *susS7* represented by each of the three intervals. This parameter (% of recombinants ÷ % of total bp) is therefore a measure of the relative intensity of exchanges per unit physical distance. This parameter is independent of the overall stimulation by Chi and reflects only the nonuniformity of the distribution of the exchanges (*i.e.*, the degree of localization of the exchanges). A value of unity for an interval indicates uniformity: the frequency of recombination (per unit physical distance) is the same in that interval as for the three intervals combined. A value above unity indicates a higher than average frequency of recombination for that interval, and a value lower than unity indicates a lower than average frequency of recombination. For the χ^- cross this parameter, called relative intensity of exchanges, was near unity for each interval, as expected if recombination in the absence of Chi is nearly uniformly distributed across λ, as reported previously from density gradient analyses of λ

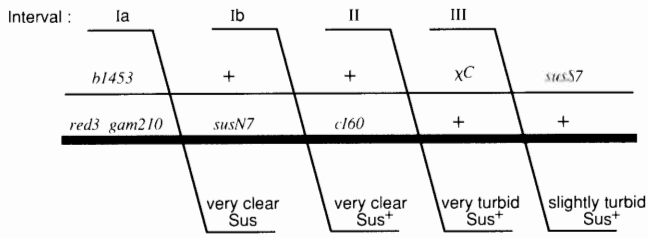

FIGURE 5.—Crosses to determine the distribution of Chi-stimulated exchange to the left of *cI60*. The crosses diagrammed here are identical to those diagrammed in Figure 4, except that the lower parent (phage 1623) contains the additional marker *susN7*. Recombinants in intervals Ia, Ib, II and III are scored as very clear Sus, very clear Sus$^+$, very turbid, and slightly turbid, respectively, as described in MATERIALS AND METHODS.

TABLE 3

Distribution of Chi-stimulated exchanges

Interval	I	II	III	Total
Interval size (bp)	4900	580	6,869	12,350
% of total bp	40	4.7	56	100
Recombinant frequency (%)				
χ^+	3.0	0.61	1.1	4.7
χ^-	0.51	0.11	1.5	2.1
Ratio of frequencies	5.9	5.5	0.73	2.2
% of b1453 S$^+$ recombinants				
χ^+	64	13	23	100
χ^-	24	6	70	100
% of recombinants ÷ % of total bp				
χ^+	1.6	2.8	0.41	
χ^-	0.6	1.3	1.3	

Crosses (Figure 4) were carried out in *sup*$^+$ *mutL*::Tn*10* strain V348 and plated on *supF recB sbcA* strain JM1 for total phage and on *supE recC1001* (P2) *mutL* strain V351 for *b1453 S*$^+$ recombinant phage as described in MATERIALS AND METHODS. For the χ^+ and χ^- crosses the total titers were 1.1×10^8 and 3.3×10^7, respectively, and the recombinant titers were 5.2×10^6 and 7.0×10^5, respectively. (All phage titers are in p.f.u. per ml of cross lysate.) For the χ^+ and χ^- crosses a total of 167 recombinant type plaques on six plates and 227 plaques on 13 plates, respectively, were analyzed for genotype as described in MATERIALS AND METHODS. Each phage type in a mixed plaque, containing heteroduplex DNA at *cI* or *cII* or both was scored as a half plaque (see Table 4). Interval sizes were calculated from the mapping data referenced in Table 2. The sizes of intervals I and II are approximate. Recombinant frequency is the titer of phage with exchanges in the indicated interval divided by the total phage titer. "% of *b1453 S*$^+$ recombinants" is the % of all selected recombinants with exchanges in the indicated interval. In the last two rows of data, these percentages are normalized for each interval's physical size (see RESULTS).

crosses (STAHL *et al.* 1974). There was a slightly higher intensity of exchanges to the right of χC than to its left, which may reflect the low level activity of sequences differing from Chi at one base pair and weakly stimulating recombination in the interval to the right of χC (CHENG and SMITH 1984). For the χ^+ cross, however, the distribution was markedly skewed to the left of Chi: the relative intensity was greatest (2.8) for the small interval immediately to the left of Chi, less (1.6) for a more distant interval to the left, and below unity (0.41) for the large interval to the right of Chi. In summary, by two measures (not entirely independent) Chi stimulated recombination in this cross to its left but not detectably to its right. (But see below for a cross with a shorter interval to the right of Chi.)

Plausible structures and frequencies of heteroduplex-containing recombinants from these crosses are shown in Table 4. The frequency of such recombinants among total phage progeny was 0.45% from the χ^+ cross and 0.10% from the χ^- cross. These heteroduplex-containing recombinants accounted for 10% of the selected *b1453 S*$^+$ recombinants from the χ^+ cross and 5% from the χ^- cross. These are minimal estimates of the frequency of hybrid DNA, since some hybrid DNA-containing recombinants could have escaped detection due to residual mismatch correction, absence of a marker at hybrid DNA regions, or loss of one or the other genotype. Such underestimations are expected to be equal, however, for the χ^+ and χ^- crosses. In subsequent crosses (see Tables 6 and 8) higher frequencies of heteroduplex-containing recombinants were also observed, by both measures, for the χ^+ crosses than for the χ^- crosses. From these results we infer that most of the heteroduplex-containing recombinants were Chi-stimulated.

The heteroduplex structures in Table 4 are defined by the markers *cI60* and χ^+C or χ^-C209 and are of two classes: I) those with one heteroduplex endpoint on each side of Chi, and II) those with both heteroduplex endpoints to the left of Chi. For the χ^+ cross class I heteroduplexes, predicted by the model in Figure 1, accounted for about 70% of the heteroduplex-containing recombinants. The class II heteroduplexes, predicted by degradation of the Chi tail, accounted for the remaining 30%.

To determine with greater precision the distribution of Chi-stimulated exchange in interval I to the left of *cI60*, crosses were done with the additional marker *susN7* in that interval (Figure 5 and Table 5). In these crosses, very clear plaques (*cI*; Figure 3) were tested for their genotype at *susN7* (pure Sus, pure Sus$^+$, or heteroduplex) as described in MATERIALS AND METHODS. The ratio of the χ^+ recombinant frequency to the χ^- recombinant frequency was greatest (4.4 and 4.1) for the intervals immediately to the left of Chi, less (2.0) for the interval farther to the left, and less than unity (0.54) for the long interval to the right of Chi. After normalization for interval size a similar pattern was seen for the relative intensity of exchange per unit physical distance in the χ^+ cross: the relative intensity was greatest immediately to the left of Chi (2.8), progressively declined farther to the left (2.0 and 1.1), and was less than unity (0.46) to the right of Chi. The intensity of exchanges in the χ^- cross was approximately constant in the four intervals. These results are in concordance with those of the previous crosses (Table 3).

Plausible structures and frequencies of heterodu-

TABLE 4

Structures and frequencies of heteroduplex-containing recombinants from the crosses of Figure 4

Structure	Scoring	Frequency per 10^4 progeny phage χ^+	Frequency per 10^4 progeny phage χ^-	Approximate Chi stimulation
Class I: One heteroduplex endpoint on each side of Chi				
b + χC / + S^+	*cII*/c^+	11 (4)	2.8 (3)	4
b + χC / *cI* + S^+	*cII*/*cI*	20 (7)	5.6 (6)	4
Total class I:		31 (11)	8.4 (9)	4
Class II: Both heteroduplex endpoints to the left of Chi				
b + / *cI* + S^+	c^+/*cI*	14 (5)	1.9 (2)	7
Total heteroduplexes:		45 (16)	10 (11)	4.5

These data comprise a subset of those in Table 3. Thin and thick lines represent DNA from upper and lower parents (Figure 4), respectively. Crosses were performed as described in Figure 4 and Table 3. For scoring see MATERIALS AND METHODS. *b*, *cI*, χC and S^+ refer to *b1453*, *cI60*, χ^+C151 (or χ^-C209) and S^+ respectively. Structures are not drawn to scale. Frequency is the titer of each heteroduplex-containing recombinant phage divided by total phage titer. Numbers of plaques observed are in parentheses. Chi stimulations (χ^+ frequency/χ^- frequency) are approximate, due to the small numbers of plaques.

TABLE 5

Distribution of Chi-stimulated exchanges to the left of *cI60*

Interval	Ia	Ib	II	III	Total
Interval size (bp)	2,200	2,700	580	6,869	12,350
% of total bp	17	22	4.7	56	100
Recombinant frequency					
χ^+	0.92	2.1	0.62	1.3	5.0
χ^-	0.45	0.48	0.15	2.4	3.6
Ratio of frequencies	2.0	4.4	4.1	0.54	1.4
% of b1453 S^+ recombinants					
χ^+	18	43	13	26	100
χ^-	13	14	4	69	100
% of recombinants ÷ % of total bp					
χ^+	1.1	2.0	2.8	0.46	
χ^-	0.8	0.6	0.9	1.2	

Crosses (Figure 5) were carried out and analyzed as discussed in MATERIALS AND METHODS and in the legend to Table 3. For the χ^+ cross and χ^- crosses, the total titers were 5.6×10^7 and 8.1×10^6 respectively, and the recombinant titers were 2.8×10^6 and 2.9×10^5, respectively. (All phage titers are p.f.u. per ml of cross lysate.) For the χ^+ and χ^- crosses a total of 445 recombinant-type plaques on 25 plates and 224 plaques on 25 plates, respectively, were analyzed.

plex-containing recombinants from these crosses are shown in Table 6. The frequency of such recombinants among total phage progeny was about five times higher from the χ^+ cross than from the χ^- cross. Of

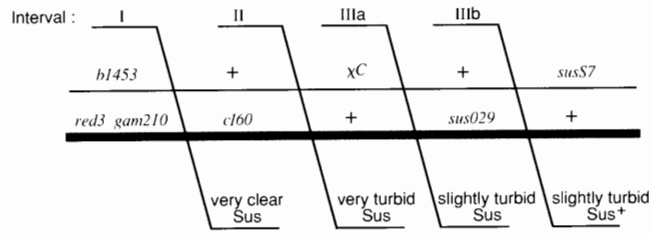

FIGURE 6.—Crosses to determine the distribution of Chi-stimulated exchange to the right of Chi. These crosses are identical to those shown in Figure 4, except that the lower parent (phage 1686) contains the marker *susO29* in addition to *cI60*. Recombinants in intervals I, II, IIIa and IIIb are scored as very clear, very turbid, slightly turbid Sus and slightly turbid Sus$^+$, respectively, as described in MATERIALS AND METHODS.

the selected *b1453* S^+ recombinants 15% were heteroduplex-containing from the χ^+ cross, and 4% from the χ^- cross. For the χ^+ cross class I heteroduplexes, with one endpoint on each side of Chi as predicted by the model in Figure 1, accounted for 40% of the total; the remainder were class II heteroduplexes, with both endpoints to the left of Chi. Recombinants heterozygous for both χ^+C and *cI60* were frequent: about one-third of the total heteroduplex-containing recombinants were of this class, the type predicted by the model in Figure 1. Heteroduplexes were occasionally detected over *susN7*, *cI60*, and χ^+C; if continuous, this heteroduplex DNA was at least 3318 bp long.

To determine with greater precision the distribution of Chi-stimulated exchange in interval III, to the right of Chi, crosses were done with the additional marker *susO29* in that interval (Figure 6 and Table 7). In these crosses all very turbid (c^+), slightly turbid (*cII*), and mottled plaques were tested for their genotype at *susO29*. The ratio of the χ^+ recombinant frequency to the χ^- frequency was greatest (23) for the short interval immediately to the left of Chi and less (13) for the interval farther to the left, in accord with the results of the previous crosses. With a short interval to the right of Chi now defined by the χ^+C and *susO29* markers, Chi stimulation was detected to the right of Chi (χ^+/χ^- ratio of 5.1). The least, and perhaps insignificant, stimulation (1.5) was observed in the interval farther to the right. A similar pattern was seen after normalizing for interval size: the relative intensity of exchange in the χ^+ cross was greatest (2.8 and 2.3) for the intervals immediately to the left and right of Chi. These intervals are of similar size (about 580 and 431 bp), and the relative intensity was about the same for both. The relative intensity for the interval farther to the left was less (1.4), and that for the interval farther to the right was less than unity (0.42). In summary, these data show that Chi stimulated recombination most strongly immediately to its left and right, less farther to its left, and perhaps insignificantly farther to its right.

For another measure of stimulation of recombina-

TABLE 6

Structures and frequencies of heteroduplex-containing recombinants from the crosses of Figure 5

Structure	Scoring	Frequency per 10^4 progeny phage χ^+	Frequency per 10^4 progeny phage χ^-	Approximate Chi stimulation
Class I: One heteroduplex endpoint on each side of Chi				
b + + χC / *+ S⁺*	*cII/c⁺* *Sus⁺*	2.3 (2)	<1.6 (0)	>1
b + + χC / *cI + S⁺*	*cI/cII* *Sus⁺*	25 (22)	3.2 (2)	8
b + + χC / *N7 cI + S⁺*	*cI/cII* *SusN* het	3.4 (3)	4.8 (3)	0.7
Total class I:		30 (27)	8 (5)	4
Class II: Both heteroduplex endpoints to the left of Chi				
b + + / *cI + S⁺*	*cI/c⁺* *Sus⁺*	11 (10)	1.6 (1)	7
b + + / *N7 cI + S⁺*	*cI/c⁺* *SusN* het	3.4 (3)	<1.6 (0)	>2
b + / *N7 cI + S⁺*	*cI* *SusN* het	30 (27)	4.8 (3)	6
Total class II:		45 (40)	6.4 (4)	7
Total heteroduplex-containing recombinants of all classes		75 (67)	14 (9)	5.4

These data comprise a subset of those in Table 5. Thin and thick lines represent DNA from upper and lower parents (Figure 5), respectively. Crosses were performed as described in Figure 5 and Table 5. Sus scoring refers to genotype at *susN7*. SusN het means heteroduplex with N^+ on one strand and *susN7* on the other. *N7* refers to *susN7*; for other abbreviations and details, see the legend to Table 4.

tion to the right of Chi, we plated the cross lysates on the *sup*$^+$ *mutL* strain V350 to select directly for O^+S^+ recombinants (interval IIIb, Figure 6). The recombinant frequencies were 5.1% and 5.2% for the χ^+ and χ^- crosses, respectively. In accord with the previous data, Chi did not detectably stimulate recombination in the interval beginning 431 bp and ending 6869 bp to its right.

Plausible structures and frequencies of heteroduplex-containing recombinants from these crosses are shown in Table 8. *susO29* to the right of Chi allows us in this case to define three classes of heteroduplexes: I) those with one heteroduplex endpoint on each side of Chi, with the right endpoint between Chi

TABLE 7

Distribution of Chi-stimulated exchanges to the right of Chi

Interval	I	II	IIIa	IIIb	Total
Interval size (bp)	4,900	580	431	6,438	12,350
% total interval	40	4.7	3.5	52	100
Recombinant frequency (%)					
χ^+	13	2.8	1.8	5.1	22
χ^-	1.0	0.12	0.35	3.3	4.8
Ratio of frequencies	13	23	5.1	1.5	4.6
% of b1453 S$^+$ recombinants					
χ^+	57	13	8	22	100
χ^-	21	2.5	7.4	69	100
% of recombinants + % of total bp					
χ^+	1.4	2.8	2.3	0.42	
χ^-	0.53	0.53	2.1	1.3	

Crosses (Figure 6) were carried out and analyzed as described in MATERIALS AND METHODS and in the legend to Table 3. For the χ^+ and χ^- crosses, the total titers were 1.7×10^7 and 2.1×10^6, respectively, and the recombinant titers were 3.7×10^6 and 1.0×10^5, respectively. (All phage titers are p.f.u./ml of cross lysate.) For the χ^+ and χ^- crosses a total of 551 recombinant-type plaques on 20 plates and 326 plaques on 20 plates, respectively, were analyzed.

and *susO29*, II) those with both heteroduplex endpoints to the left of Chi, and III) those with heteroduplex spanning both Chi and rightward marker *susO29*. In the previous crosses recombinants of the third class would have been included among class I heteroduplexes. In contrast to the results of the previous crosses, a large number of multiple exchange recombinants were detected. We inferred the continuous heteroduplex DNA structures (class IIIb) that would account for the multiple exchange recombinants by mismatch correction. Mismatch correction occurs in *mutL* mutant hosts for some nonsense markers in DNA replication genes, such as *susO29*, when heteroduplex phage are analyzed under restrictive conditions (RADICELLA, CLARK and FOX 1988); such mismatch correction may have altered the heteroduplexes in our crosses. This possibility renders the conclusions about the heteroduplexes from this third set of crosses somewhat uncertain.

The frequency of heteroduplex-containing recombinants among total progeny phage was about 18 times higher for the χ^+ cross than for the χ^- cross. Among the selected *b1453* S^+ recombinants 8% were heteroduplex-containing from the χ^+ cross and 2% from the χ^- cross. In the χ^+ cross, classes I, II and III comprised about 15, 53, and 32% of the heteroduplex-containing recombinants, respectively, not counting any inferred (class IIIb) heteroduplex-containing recombinants. Counting class IIIb recombinants with observed heteroduplex, the percentages were about 11, 41 and 48%, respectively. Class I heteroduplexes, with one endpoint in the short interval immediately to the right of Chi as predicted by the model in Figure 1, therefore comprised a significant fraction of the observed heteroduplex-containing recombinants. The other two classes can be accounted

TABLE 8

Structures and frequencies of heteroduplex-containing recombinants from the crosses of Figure 6

Structure	Scoring	Frequency per 10^4 progeny phage χ^+	χ^-	Approximate Chi stimulation
Class I: One heteroduplex endpoint immediately to the right of Chi, the other to the left of Chi				
b + χ*C* / + *O29* S^+	*cII*/c^+ *SusO*	8 (2)	<1.5 (0)	>5
b + χ*C* / *cI* + *O29* S^+	*cII*/*cI* *SusO*	12 (3)	1.5 (1)	8
Total class I:		20 (5)	1.5 (1)	>10
Class II: Both heteroduplex endpoints to the left of Chi				
b + / *cI* + *O29* S^+	c^+/*cI* *SusO*	73 (18)	<1.5 (0)	>50
Class IIIa: Definite heteroduplex at both χC and *susO29*				
b + χ*C* + / + *O29* S^+	*cII*/c^+ *SusO* het	12 (3)	3 (2)	4
b + χ*C* + / *cI* + *O29* S^+	*cII*/*cI* *SusO* het	32 (8)	3 (2)	10
Total class IIIa:		45 (11)	6 (4)	8
Class IIIb: Inferred heteroduplexes at both χC and *susO29*, mismatch-corrected at one or more sites				
b + (χ*C*) + / + *O29* S^+	c^+ *SusO* het	4 (1)	<1.5 (0)	>2
b + χ*C* + / *cI* + (*O29*) S^+	*cII*/*cI* Sus^+	16 (4)	1.5 (1)	10
b + (χ*C*) + / *cI* + (*O29*) S^+	c^+/*cI* Sus^+	4 (1)	<1.5 (0)	>2
b (+) (χ*C*) + / *cI* + (*O29*) S^+	*cI* Sus^+	61 (15)	4 (3)	15
b (+) (χ*C*) + / *cI* + *O29* S^+	*cI* *SusO* het	16 (4)	1.5 (1)	10
Total class IIIb:		100 (25)	7 (5)	14
Total classes IIIa and IIIb:		150 (36)	13 (9)	12
Total heteroduplex-containing recombinants of all classes[a]		180 (44)	10 (7)	18

These data comprise a subset of those in Table 7. Thin and thick lines represent DNA from upper and lower parents (Figure 6), respectively. Crosses were performed as described in Figure 6 and Table 7. Markers in parentheses were inferred to have been in the heteroduplex before mismatch correction. *Sus* scoring refers to genotype at *susO29*. *SusO* het means heteroduplex with O^+ on one strand and *susO29* on the other. *O29* refers to *susO29*; for other abbreviations and details see legend to Table 4.

[a] Among the class IIIb recombinants, only *cI* Sus^+ plaques were not truly scored as heteroduplexes; their generation can be either via mismatch correction of the structure drawn or by multiple exchanges (intervals I, IIIa and IIIb). These were not included in the calculation of total heteroduplex-containing recombinants of all classes.

for by the modifications of the model suggested in the DISCUSSION.

Table 9 summarizes the data from all three crosses for comparison. Nearly all of the measured parameters were reproducible from cross to cross. The exception was the higher frequency of recombinants in the χ^+ cross of Figure 6. The basis of this difference is not known, but after normalization for interval sizes, the relative intensities of exchange from this cross agree with those from the other crosses. The difference may simply be due to a lower total phage yield determination for the χ^+ cross of Figure 6.

Figure 7 displays the distribution of exchanges in the presence of Chi for the crosses diagrammed in Figures 4, 5 and 6. We graphed the relative frequency of exchange per unit physical distance for each interval *vs.* the position of each interval relative to Chi. The data indicate an exponential decrease in the frequency of exchange to the left of Chi, significant exchange immediately to the right of Chi, and only "background" exchange (*i.e.*, equivalent to that in the absence of Chi) farther to the right of Chi.

Chi reproducibly stimulated the appearance of heteroduplex DNA among the recombinants (Table 9). Frequently, one endpoint of this heteroduplex was to the right of Chi, and the other was to the left of Chi (Tables 4, 6 and 8).

We sought mutant bacteria in which the distribution of Chi-stimulated exchange was altered. Alteration in the distribution due to elimination or overproduction of enzymes known or suspected to be important in recombination would shed light on the mechanism by which Chi stimulates recombination. The crosses diagrammed in Figure 5, with four scorable intervals, were conducted in and analyzed on Mut^+ hosts; recombinants heteroduplex at *susN7* were not distin-

TABLE 9

Summary of data for the crosses in Figures 4, 5, and 6

Interval	I		II		III	
Recombinant frequency (%)						
Crosses in Figure	x^+	x^-	x^+	x^-	x^+	x^-
4	3.0	0.51	0.61	0.11	1.1	1.5
5	3.0	0.93	0.62	0.15	1.3	2.4
6	13	1.0	2.8	0.12	6.9	3.7
% of b1453 S^+ recombinants						
Crosses in Figure	x^+	x^-	x^+	x^-	x^+	x^-
4	64	24	13	6.0	23	70
5	61	27	13	4.0	26	69
6	57	21	13	2.5	30	76
% of recombinants ÷ % of total bp						
Crosses in Figure	x^+	x^-	x^+	x^-	x^+	x^-
4	1.6	0.60	2.8	1.3	0.41	1.3
5	1.6	0.69	2.8	0.9	0.46	1.2
6	1.4	0.53	2.8	0.53	0.54	1.4

	Total het frequency (%)			% het among b1453 S^+ recombinants		
Crosses in Figure	x^+	x^-	Ratio	x^+	x^-	Ratio
4	0.45	0.10	4.5	9.6	4.8	2.0
5	0.75	0.14	5.4	15.0	4.0	3.8
6	1.8	0.10	18	8.2	2.1	3.9

The data are derived from Tables 3–8 and shown here to demonstrate the reproducibility from cross to cross. For the crosses in Figure 5, the data for intervals Ia and Ib are combined; for the crosses in Figure 6, the data for intervals IIIa and IIIb are combined.

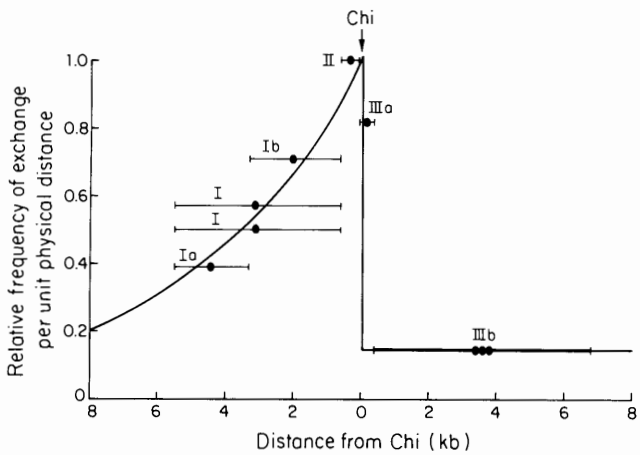

FIGURE 7.—Distribution of recombinational exchanges in the presence of Chi. The relative frequency of exchange per unit physical distance is proportional to the relative intensity of recombinational exchanges for each interval (% of recombinants ÷ % of total base pairs; Tables 3, 5 and 7). The endpoints of each line segment denote the positions, relative to Chi, of the markers defining each interval measured (Figures 4, 5 and ,6). The point in the middle of each segment indicates the frequency of exchange per unit physical distance, normalized by setting that for interval II equal to 1. The solid line indicates the distribution predicted by the model in Figure 1, with a 7-fold Chi-stimulation near Chi and an exponential distribution of exchanges to the left of Chi decreasing 2-fold for each 3.2 kb.

guished from N^+ recombinants since they made only a minor contribution. The following nuclease mutants showed no significant change in the distribution of Chi-stimulated exchange in such crosses (data not shown): the 3′→5′ single strand exonuclease I (product of *sbcB;* KUSHNER *et al.* 1971; YAJKO, VALENTINE and WEISS 1974), the bidirectional single strand exonuclease ExoVII (product of *xseA* and *xseB;* CHASE and RICHARDSON 1977; VALES, RABIN and CHASE 1983), and the 3′→5′ proofreading exonuclease functions of PolI (*polA1*ex$^-$; KORNBERG 1980) and PolIII (ε-subunit, product of *dnaQ*; SCHEUERMANN and ECHOLS 1984; SCHEUERMANN *et al.* 1983). Crosses in strains containing plasmids overproducing RecBCD enzyme (PONTICELLI *et al.* 1985), the λ Gam protein (FRIEDMAN and HAYS 1986), RecA protein (MCENTEE 1977), Ssb protein (R. MCMACKEN, personal communication), exonuclease I or DnaQ protein also showed no significant differences (data not shown).

DISCUSSION

To test a model of Chi-stimulated recombination (Figure 1), phage lambda crosses (Figures 4, 5 and 6) were performed in hosts with impaired mismatch correction. Overall, the results (Tables 3, 5, 7, and summarized in Table 9 and Figure 7) show, by two measures, that Chi stimulation was greatest in the intervals immediately to the right and left of Chi. Stimulation was not detectable farther to the right (more than 431 bp to the right of Chi). Stimulation decreased exponentially with distance to the left: a 2-fold decrease per 3.2 kb. This distance-dependence was similar to that reported by ENNIS, AMUNDSEN, and SMITH (1987), a 2-fold decrease per 2.2 kb for crosses employing large heterologies. (The crosses reported here employed only single base pair mutations in the region where recombination was measured.) As inferred from heterozygous phage particles, Chi stimulated the formation of heteroduplex DNA, both at itself and to its left (Tables 4, 6 and 8). A frequently observed class of heteroduplexes had one endpoint to the right of Chi and the other endpoint to the left of Chi.

These observations support the model in the top part of Figure 1. The DNA strand cleavage by RecBCD enzyme about five nucleotides to the right of Chi (step D) accounts for the Chi-stimulated exchanges observed in the interval immediately to the right of Chi. The RecA and SSB protein-promoted synapsis of the single-stranded DNA tail containing the Chi sequence with a homologous duplex DNA (step F) accounts for the genetic heterozygosity at Chi and to its left observed in Chi-stimulated recombinants. The cleavages of the Holliday junctions exponentially distributed to the left of Chi (step H) account for the Chi-stimulated exchanges observed with diminishing frequency to the left of Chi.

Two findings call for a minor modification of the model in the top part of Figure 1. First, more than half of the Chi-stimulated exchanges were to the left of Chi (Tables 3, 5 and 7). Second, Chi stimulated the formation of recombinants containing heteroduplex DNA far to the left of Chi but not covering Chi (class II heteroduplexes, Tables 4, 6 and 8). These findings are readily accommodated by degradation of the single-stranded DNA containing Chi ("Chi tail," step F in Figure 1) more than about 10 nucleotides before synapsis (step F'). The resultant recombinants would not be heteroduplex at Chi, and the points of strand exchange would be to the left of Chi (step I', Figure 1). Chi tail degradation cannot occur every time, however, since cass I heteroduplexes, which are heteroduplex at Chi and are inconsistent with such degradation, make up a significant proportion of the detected heteroduplex-containing recombinants. Furthermore, we observed significant stimulation of exchange immediately to the right of Chi, which in the context of the model in Figure 1 requires the Chi tail to remain intact.

The most sensitive estimate we have of the frequency of degradation of the Chi tail derives from the Figure 5 crosses, where two markers to the left of Chi permit detection of heteroduplex DNA at those markers. About 60% of the heteroduplex-containing recombinants from the χ^+ cross had both heteroduplex endpoints to the left of Chi (Table 6), implying that, in the context of the model in Figure 1, about 40% of the Chi tails remained intact. Consideration of the fraction of all exchanges to the right and left of Chi (not just those with detected heteroduplex) gives a similar estimate of the fraction of the Chi tails that remain intact. Of the exchanges in the χ^+ crosses, 23% to 30% were to the right of Chi (Table 9; CHENG and SMITH 1984). In summary, the Chi tail appears to remain intact about 25% to 40% of the time. (Degradation of the Chi tail less than 10 nucleotides, from its 3′ end to the χ^+*C151* mutation, could not have been detected.)

Those instances in which the Chi tail is degraded can account for Chi's action to its left when opposite a large heterology (STAHL and STAHL 1975; STAHL *et al.* 1980) and for localized DNA synthesis in the vicinity of Chi (SIEGEL 1974) (see bottom part of Figure 1). After degradation of the Chi tail through the region of heterology into the region of homology, strand invasion by the resultant single-stranded DNA 3′-end can be promoted by RecA and SSB proteins (KONFORTI and DAVIS 1987). Degradation past the heterology boundary, or in the absence of any heterology, would leave a single-stranded DNA gap which could be filled by DNA polymerase. This gap filling can account for the localized DNA synthesis near Chi in a fully homologous "cross" (SIEGEL 1974), the occurrence of some recombinants without detectable heteroduplex at or near Chi (Tables 4, 6 and 8), and the requirement for DNA polymerase I for Chi-stimulated recombination (ENNIS, AMUNDSEN, and SMITH 1987).

The model in Figure 1 predicts that recombinants parental for flanking markers (Figure 1, I left) initially contain heteroduplex with the single strand insertion on the *l* strand of λ (the strand with its 3′ end on the right). ROSENBERG (1987) reported that the single-strand insertion is on the *r* strand. The genetic factor responsible for this strand bias in the observed heteroduplexes has not been reported, but neither Chi nor *cos* (λ's cohesive end site) appear responsible (ROSENBERG 1987, 1988). Most (about 75%) of the recombinants parental for flanking markers reported in her studies were homoduplex at the monitored *susP80* marker, whether DNA replication was blocked or not. The formation of those homoduplex recombinants may have involved mismatch correction, which may have abolished heteroduplexes with a single-strand insertion of the *l* strand. Mismatch correction occurs in *mutL* mutant hosts, as ROSENBERG (1987, 1988) used, for some nonsense markers in DNA replication genes, such as *susP80,* when heteroduplex phage are analyzed under restrictive conditions (RADICELLA, CLARK and FOX 1988). In the heteroduplexes reported here, it was not possible to test for strand bias since the heteroduplexes were necessarily replicated during plaque formation.

A minority of the recombinants studied here were heteroduplex at a marker *(susO29)* located 431 bp to the right of Chi (Table 8). This class of recombinants is not predicted by the models in Figure 1. These Chi-stimulted recombinants may have arisen from synapsis of the 5′ ended single-stranded DNA tail to the right of Chi (Figure 1, step D) with homologous DNA or from more complex events. SMITH *et al.* (1984) proposed an alternative model for Chi-stimulated recombination in which invasion by a degraded 3 Chi tail leads to heteroduplex DNA spanning Chi and markers both to its left and to its right. That model can account for the minority recombinants observed here.

To identify the nuclease postulated to degrade the Chi tail, crosses were conducted in mutants in which nucleases were either defective or overproduced (some candidates are reviewed by WEISS 1981). Increased Chi tail degradation would shift the distribution of Chi-stimulated exchanges to the left, whereas decreased degradation would shift the distribution to the right. Significant shifts were not observed in the nuclease mutants and overproducers tested (see RESULTS). The Chi tail may be degraded by the Chi-independent nuclease activity of RecBCD enzyme (TAYLOR 1988). Testing this possibility is difficult, since eliminating RecBCD enzyme eliminates Chi ac-

tivity, leaving no Chi-stimulation to measure. Overproducing RecBCD enzyme may have no effect because one enzyme molecule may both cut at Chi and degrade the tail; the enzyme is highly processive (TAYLOR 1988). Further experiments are required to identify the postulated Chi-tail nuclease.

We thank our colleagues SUE AMUNDSEN, GARY BRAEDT, ABED CHAUDHURY, SUE HOLBECK, NANCY HOLLINGSWORTH, KAREN LARSON, LARRY LOEB, FRED PONTICELLI, DENNIS SCHULTZ, PHILIPPE SZANKASI, ANDREW TAYLOR and STAN BROWN for support and discussion. We thank FRANK and MARY STAHL for valuable discussions and for communicating unpublished observations. DENNIS SCHULTZ and ANDREW TAYLOR generously contributed *E. coli* and λ strains and technical advice as well. *E. coli* and λ strains and information about them were kindly shared by BARBARA BACHMANN, JOHN CHASE, MAURICE FOX, SIDNEY KUSHNER, KEVIN MCENTEE, ROGER MCMACKEN, PABLO RADICELLA and RICHARD SCHEUERMANN. This work was supported by U.S. Public Health Service grant GM31693 to G.R.S, and by U.S. Public Health Service training grant T32 GM07187 and Damon Runyon-Walter Winchell Cancer Research Fund Fellowship DRG-O49 to K.C.C.

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Communicating editor: G. MOSIG