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

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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 synopsis with homologous DNA.


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 1988b), 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 imm21 stimulated exchange in the interval imm21-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...
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<sup>-</sup> Gam<sup>-</sup> 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 mut<sup>L</sup> 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 OT<sup>P</sup><sub>+</sub> recombinants heteroduplex at cl is 10–20-fold higher when the cross lysate is plated on mut<sup>L218::Tn10</sup>, rather than mut<sup>L+</sup>, indicator bacteria;
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 \( \chi^+ \) 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 (\( dl \)); 3. mottled (\( dl/c^+ \)); 4. slightly turbid (\( dl \)).

c\( l 60 \) (Kaiser 1957) and \( \chi^+C \), which is a recombinational hotspot (Stahl, Crasemann and Stahl 1975) as well as a plaque morphology marker in \( dl \). Thus, recombinants heteroduplex for either or both of these markers were detected as mottled plaques (Figure 3) on \( mutL::Tn10 \) 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 \( \lambda \) 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 "\( \chi^- \) cross" containing the inactive \( \chi^-C209 \) 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 \( \chi^+ \) cross.

### 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 (Stiegel 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 \( \mu \)g/ml thiamine, and 2.5 mM MgSO\(_4\). BBL agar is 1% Trypticase peptone (BBL, Cockeysville, MD), 1% 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), 1% NaCl, and 1.0% Trypticase peptone (BBL). BBL-YE agar contains 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\(^9\) 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\(^9\) plating bacteria freshly grown

### TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>V322</td>
<td>mutL218::Tn10 supF argE(am)</td>
<td>HUSMAN and FOX</td>
</tr>
<tr>
<td></td>
<td>mutB gyrA(Nal(^R)) rpsB(Rif(^R)) de(lac-pro)</td>
<td>(1986) strain D6432</td>
</tr>
<tr>
<td>594</td>
<td>lac-3350 galK2 galF2 rpsL179</td>
<td>WEIGLE (1966)</td>
</tr>
<tr>
<td>V348</td>
<td>as 594 plus mutL218::Tn10</td>
<td>V322 x 594(*)</td>
</tr>
<tr>
<td>V228</td>
<td>supE recC1001 argA his gal</td>
<td>D. W. SCHULTZ</td>
</tr>
<tr>
<td></td>
<td>endA tonA</td>
<td>(strain D241)</td>
</tr>
<tr>
<td>V227</td>
<td>as V228 plus (P2)</td>
<td>Lysogen of V228</td>
</tr>
<tr>
<td>V351</td>
<td>as V228 plus mutL218::Tn10</td>
<td>V322 x V227</td>
</tr>
<tr>
<td>JCS879</td>
<td>recB21 recC22 shaA23 supF44</td>
<td>GILLEN (1974)</td>
</tr>
<tr>
<td></td>
<td>thr-1 leu-6 thi-1 lacY1 galK2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ara-14 xyl-5 proA2 his-4 argE3 rpsL31 tss-33 mil-1</td>
<td></td>
</tr>
<tr>
<td>JCS987</td>
<td>recB21 recC22 shaB15 thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 proA2 his-4 argE3 rpsL31 tss-33 mil-1</td>
<td>GILLEN (1974)</td>
</tr>
<tr>
<td>V350</td>
<td>as JCS987 plus mutL218::Tn10</td>
<td>V322 x JCS987</td>
</tr>
<tr>
<td>JM1</td>
<td>recB21 shaA20 supF his-318</td>
<td>STAHL et al. (1980)</td>
</tr>
<tr>
<td></td>
<td>leu(am) lac(ami)</td>
<td></td>
</tr>
</tbody>
</table>

* A x B, P1 transduction where A is the donor and B is the recipient. Also see MATERIALS AND METHODS.
TABLE 2
Phage lambda strains used

<table>
<thead>
<tr>
<th>Strain</th>
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</thead>
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<tr>
<td>1429</td>
<td>b1453 χ’C151</td>
</tr>
<tr>
<td>1503</td>
<td>red-3 gam-210 cl60</td>
</tr>
<tr>
<td>1556</td>
<td>b1453 χ’C151 χ’C209</td>
</tr>
<tr>
<td>1568</td>
<td>b1453 χ’C151 sus7</td>
</tr>
<tr>
<td>1569</td>
<td>b1453 χ’C151 χ’C209 sus7</td>
</tr>
<tr>
<td>1570</td>
<td>b1453 cl60</td>
</tr>
<tr>
<td>1581</td>
<td>b1453</td>
</tr>
<tr>
<td>1623</td>
<td>red-3 gam-210 susN7 cl60</td>
</tr>
<tr>
<td>1647</td>
<td>b1453 susN7 cl60</td>
</tr>
<tr>
<td>1650</td>
<td>b1453 susO29</td>
</tr>
<tr>
<td>1651</td>
<td>b1453 χ’C151 susO29</td>
</tr>
<tr>
<td>1652</td>
<td>b1453 χ’C151 χ’C209 susO29</td>
</tr>
<tr>
<td>1686</td>
<td>red-3 gam-210 cl60 susO29</td>
</tr>
</tbody>
</table>

Sources of the alleles are as follows: b1453, Henderson and Weil (1975); χ’C151, Stahl, Crasemann and Stahl (1975); susN7, Goldberg and Howe (1969); χ’C209, Schultz, Swindle and Smith (1981); red-3, Signer and Weil (1968); gam-210, Zisler, Signer and Schafer (1971a); supN7 and susO29, Campbell (1961); cl60, 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 Weil (1975); for our calculations the right endpoint is assumed to be in the middle of the gene. cl60 is in the right part of cl (Smith 1975), and for our calculations is assumed to be 10% from the right end.

STB to about 5 x 10^10 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’ cross.

The basis of the selection for recombinants for flanking markers is as follows: gam-210 is suppressed by supN7 (Zisler, Signer and Schafer 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 (Zisler, Signer and Schafer 1971b), and supS7 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 1988).

For the crosses in Figures 4 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; plaques 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 restreaked by picking with a fine platinum needle with a spherical end and streaking on lawns of the supE recC1001 (P2) strain V551 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 mixed 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 cl or cl’ 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 (cl) 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 cl recombinant was scored as N+ on both strands. When one or more of the step 2 plaques were Sus, the original cl recombinant was scored as N+/susN7 heteroduplex.

To test for plaques mixed at susO29 among very turbid (e’) and slightly turbid (cl) 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 30 restratifications 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.

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Chi-Stimulated Exchange Distribution

![Diagram](image)

**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 \( \chi^+ C131 \) (Chi, 5' G-C-T-G-G-T-G-G-3') at \( \chi^c \), and in the other, the upper parent (phage 1569) contains the \( \chi^+ C209 \) octamer (5' G-C-T-G-G-T-A-G-G-3') at \( \chi^C \) (underlined bases are changes from wild type) (SCHULTZ, SWINDELE 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 1). Crosses were done as described in MATERIALS AND METHODS. The basis of the segregation scoring for genetic exchange in the three crosses, and scoring for mottled (heteroduplex-containing) plaques are also described in MATERIALS AND METHODS. Distances between markers are not drawn to scale.

First, \( \chi^+ \) and \( \chi^- \) "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 \( \chi^+ \) 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 \( cl \) and \( dll \) genotypes, 10 \( cl \) (very clear) and 8 \( dll \) (slightly turbid) recombinant plaques, and 18 \( cl \) and 6 \( dll \) plaques from streakings of mixed plaques from the Figure 4 \( \chi^+ \) cross, were tested for complementation by spotting with known \( cl \) or \( cl \) phage (KAISER 1957). All complementation results were consistent with the initial scorings. No \( cl dll \) double mutants (expected to be \( cl \) by visual scoring) were observed.

**RESULTS**

Three sets of \( \lambda \) Red+ Gam- crosses were analyzed to determine the distribution of Chi-stimulated ex-
and below unity all selected recombinants with exchanges in the indicated interval.

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 cl60 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 cl60, crosses were done with the additional marker susN7 in that interval (Figure 5 and Table 5). In these crosses, very clear plaques (cl; 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

<table>
<thead>
<tr>
<th>Structure</th>
<th>Scoring</th>
<th>Frequency per 10^4 progeny phage</th>
<th>Approximate Chi stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b + \chi C + S*</td>
<td>cll/c+</td>
<td>11 (4)</td>
<td>2.8 (3)</td>
</tr>
<tr>
<td>cl + S*</td>
<td>cll/c+</td>
<td>20 (7)</td>
<td>5.6 (6)</td>
</tr>
<tr>
<td>Total class I:</td>
<td></td>
<td>31 (11)</td>
<td>8.4 (9)</td>
</tr>
<tr>
<td>Class II: Both heteroduplex endpoints to the left of Chi</td>
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<td></td>
<td></td>
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<tr>
<td>b + cl</td>
<td>c+cl</td>
<td>14 (5)</td>
<td>1.9 (2)</td>
</tr>
<tr>
<td>cl + S*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total heteroduplexes:</td>
<td></td>
<td>45 (16)</td>
<td>10 (11)</td>
</tr>
</tbody>
</table>

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, cl, \chi C, and S* refer to b1453, cl60, \chi C151 (or \chi 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 (\chi^+ frequency/\chi^- frequency) are approximate, due to the small numbers of plaques.

TABLE 5
Distribution of Chi-stimulated exchanges to the left of cl60

<table>
<thead>
<tr>
<th>Interval</th>
<th>Ia</th>
<th>Ib</th>
<th>II</th>
<th>III</th>
<th>Total</th>
<th>% of total bp</th>
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<td>Recombinant frequency</td>
<td>\chi^+</td>
<td>0.92</td>
<td>2.1</td>
<td>0.62</td>
<td>1.3</td>
<td>5.0</td>
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<td></td>
<td>\chi^-</td>
<td>0.45</td>
<td>0.48</td>
<td>0.15</td>
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<td>3.6</td>
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<td>Ratio of frequencies</td>
<td>2.0</td>
<td>4.4</td>
<td>4.1</td>
<td>0.54</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>% of b1453 S* recombinants</td>
<td>\chi^+</td>
<td>18</td>
<td>43</td>
<td>13</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>\chi^-</td>
<td>13</td>
<td>14</td>
<td>4</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td>% of recombinants + % of total bp</td>
<td>\chi^+</td>
<td>1.1</td>
<td>2.0</td>
<td>2.8</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>\chi^-</td>
<td>0.8</td>
<td>0.6</td>
<td>0.9</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

Crossovers (Figure 5) were carried out and analyzed as discussed in MATERIALS AND METHODS and in the legend to Table 3. For the \chi^+ cross and \chi^- crosses, the total titers were 5.6 \times 10^6 and 8.1 \times 10^6 respectively, and the recombinant titers were 2.8 \times 10^6 and 2.9 \times 10^6, respectively. (All phage titers are p.f.u. per ml of cross lysate.) For the \chi^+ and \chi^- 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 \chi^+ cross than from the \chi^- cross. Of the selected b1453 S* recombinants 15% were heteroduplex-containing from the \chi^+ cross, and 4% from the \chi^- cross. For the \chi^- 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 \chi C and cl60 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, cl60, and \chi 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 (cl), and mottled plaques were tested for their genotype at susO29. The ratio of the \chi^+ recombinant frequency to the \chi^- 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 shorter interval to the right of \chi now defined by the \chi C and susO29 markers, Chi stimulation was detected to the right of \chi (\chi^+/\chi^- 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 \chi^+ 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 recombinant...
plex-containing recombinants from these crosses are between Chi, with the right endpoint between Chi 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 \( \chi^+ \) cross than for the \( \chi^- \) cross. Among the selected \( b1453 \) \( S^+ \) recombinants 8% were heteroduplex-containing from the \( \chi^+ \) cross and 2% from the \( \chi^- \) cross. In the \( \chi^+ \) 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>
<thead>
<tr>
<th>Structure</th>
<th>Scoring</th>
<th>Frequency per 10^4 progeny phage</th>
<th>Approximate Chi stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I: One heteroduplex endpoint on each side of Chi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( b + + \chi C )</td>
<td>cl/c^+</td>
<td>( 2.3 &lt; 1.6 )</td>
<td>&gt;1</td>
</tr>
<tr>
<td>( + S^+ )</td>
<td>Sus^+</td>
<td>(2) (0)</td>
<td></td>
</tr>
<tr>
<td>( b + + \chi C )</td>
<td>cl/c^+</td>
<td>( 25 &lt; 1.6 )</td>
<td>8</td>
</tr>
<tr>
<td>( + S^+ )</td>
<td>Sus^+</td>
<td>(22) (2)</td>
<td></td>
</tr>
<tr>
<td>( b + + \chi C )</td>
<td>cl/c^+</td>
<td>( 3.4 &lt; 1.6 )</td>
<td>0.7</td>
</tr>
<tr>
<td>( N7 cl + S^+ )</td>
<td>SusN het</td>
<td>(3) (3)</td>
<td></td>
</tr>
<tr>
<td>Total class I:</td>
<td></td>
<td>30 8</td>
<td>(27) (5)</td>
</tr>
<tr>
<td>Class II: Both heteroduplex endpoints to the left of Chi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( b + + )</td>
<td>cl/c^+</td>
<td>( 11 &lt; 1.6 )</td>
<td>7</td>
</tr>
<tr>
<td>( cl + S^+ )</td>
<td>Sus^+</td>
<td>(10) (1)</td>
<td></td>
</tr>
<tr>
<td>( b + + )</td>
<td>cl/c^+</td>
<td>( 3.4 &lt; 1.6 )</td>
<td>&gt;2</td>
</tr>
<tr>
<td>( N7 cl + S^+ )</td>
<td>SusN het</td>
<td>(3) (0)</td>
<td></td>
</tr>
<tr>
<td>( b + + )</td>
<td>cl</td>
<td>( 30 &lt; 1.6 )</td>
<td>7</td>
</tr>
<tr>
<td>( N7 cl + S^+ )</td>
<td>SusN het</td>
<td>(27) (3)</td>
<td></td>
</tr>
<tr>
<td>Total class II:</td>
<td></td>
<td>45 6.4</td>
<td>(40) (4)</td>
</tr>
<tr>
<td>Total heteroduplex-containing recombinants of all classes</td>
<td></td>
<td>75 14</td>
<td>(67) (9)</td>
</tr>
</tbody>
</table>

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.
### TABLE 8

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

<table>
<thead>
<tr>
<th>Structure</th>
<th>Scoring</th>
<th>Frequency per 10^4 progeny phage</th>
<th>Approximate Chi stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I: One heteroduplex endpoint immediately to the right of Chi, the other to the left of Chi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b + $\chi$C</td>
<td>susO cl</td>
<td>8</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>cl + O29 S$^+$</td>
<td></td>
<td>(2)</td>
<td>(1)</td>
</tr>
<tr>
<td>b + $\chi$C</td>
<td>susO cl</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>cl + O29 S$^+$</td>
<td></td>
<td>(3)</td>
<td>(1)</td>
</tr>
<tr>
<td>Total class I:</td>
<td></td>
<td>20</td>
<td>(5)</td>
</tr>
<tr>
<td>Class II: Both heteroduplex endpoints to the left of Chi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b +</td>
<td>c$^+$</td>
<td>73</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>cl + O29 S$^+$</td>
<td></td>
<td>(18)</td>
<td>(0)</td>
</tr>
<tr>
<td>Class IIIa: Definite heteroduplex at both $\chi$C and susO29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b + $\chi$C</td>
<td>c$^+$</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>susO het</td>
<td></td>
<td>(3)</td>
<td>(2)</td>
</tr>
<tr>
<td>b + $\chi$C</td>
<td>cl</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>susO het</td>
<td></td>
<td>(8)</td>
<td>(2)</td>
</tr>
<tr>
<td>Total class IIIa:</td>
<td></td>
<td>45</td>
<td>(11)</td>
</tr>
</tbody>
</table>

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.

* Among the class IIIb recombinants, only cl 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 $\chi^-$ 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 $\chi^+$ 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-
binational exchanges
exponential distribution of exchanges to the left of Chi decreasing
unit physical distance, normalized by setting that for interval
equal to segment denote the positions, relative to Chi, of the markers defin-
middle of each segment indicates the frequency of exchange per
unit physical distance is proportional to the relative intensity of recom-
model in Figure 2-fold
presence of Chi. The relative frequency of exchange per unit
interval measured (Figures 4, 5 and 6). The data are derived from Tables 3, 5, 7, and sum-
area 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 communica-
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 synopsis 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.

![Figure 7](image-url)

**TABLE 9**
Summary of data for the crosses in Figures 4, 5, and 6

<table>
<thead>
<tr>
<th>Interval</th>
<th>I (%)</th>
<th>II (%)</th>
<th>III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant frequency (%)</td>
<td>x+ x- x+ x- x+ x-</td>
<td>x+ x- x+ x- x+ x-</td>
<td></td>
</tr>
<tr>
<td>Crosses in Figure 4</td>
<td>3.0 0.51 0.61 0.11 1.1 1.5</td>
<td>3.0 0.93 0.62 0.15 1.3 2.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>13 1.0 2.8 0.12 6.9 3.7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>% of b1453 S* recombinants</td>
<td>x+ x- x+ x- x+ x-</td>
<td>x+ x- x+ x- x+ x-</td>
<td></td>
</tr>
<tr>
<td>Crosses in Figure 4</td>
<td>64 24 13 6.0 23 70</td>
<td>61 27 13 4.0 26 69</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>57 21 13 2.5 30 76</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>% of recombinants + % of total bp</td>
<td>x+ x- x+ x- x+ x-</td>
<td>x+ x- x+ x- x+ x-</td>
<td></td>
</tr>
<tr>
<td>Crosses in Figure 4</td>
<td>1.6 0.60 2.8 1.3 0.41 1.3</td>
<td>1.6 0.69 2.8 0.9 0.46 1.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.4 0.53 2.8 0.53 0.54 1.4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total het frequency (%)</td>
<td>% het among b1453 S* recombinants</td>
<td>x+ x-</td>
<td></td>
</tr>
<tr>
<td>Crosses in Figure 4</td>
<td>x+ x- Ratio x+ x- Ratio</td>
<td>x+ x-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.45 0.10 4.5 9.6 4.8 2.0</td>
<td>0.75 0.14 5.4 15.0 4.0 3.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.8 0.10 18 8.2 2.1 3.9</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
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 class 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-stimulated 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. Over-
producing RecBCD enzyme may have no effect be-
cause one enzyme molecule may both cut at Chi and
degrade the tail; the enzyme is highly processive (TA-
YOR 1988). Further experiments are required to iden-
tify the postulated Chi-tail nuclease.

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Winchell Cancer Research Fund Fellowship DRG-O49 to K.C.C.

LITERATURE CITED

Brae, G. and G. R. Smith, 1989 Strand specificity of DNA
unwinding by RecBCD enzyme. Proc. Natl. Acad. Sci. USA
86: 871–875.

Campbell, A., 1961 Sensitive mutants of bacteriophage λ. Viro-

Chase, J. W., and C. C. Richardson, 1977 Escherichia coli 

Cheng, K. C., and G. R. Smith, 1984 Recombinational hotspot

Cheng, K. C., and G. R. Smith, 1987 Cutting of Chi-like se-

Clark, J. A., 1973 Recombination deficient mutants of E. coli 

Cox, M. M., and I. R. Lehman, 1987 Enzymes of general recom-

functions promoting homologous recombination in Escherichia 

Friedman, S. A., and J. B. Sivas, 1986 Selective inhibition of 
Escherichia coli RecBC enzyme functions by plasmid-encoded 

Gillen, J. R., 1974 The RecE pathway of genetic recombina-
tion in Escherichia coli. Ph.D. thesis, University of California, Berkeley,
Calif.

Goldberg, A. R., and M. Howe, 1969 New mutations in the S 
cistron of bacteriophage lambda affecting host cell lysis. Virol-

Henderson, D., and J. Weil, 1975 Recombination-deficient dele-
tions in bacteriophage lambda and their interaction with chi 

Huisman, O., and M. S. Fox, 1986 A genetic analysis of primary 
products of bacteriophage lambda recombination. Genetics

Kaiser, A. D., 1957 Mutations in a temperate bacteriophage 
favoring its ability to lysogenize Escherichia coli. Virology 3:
41–61.

Konforti, B. B., and R. W. Davis, 1987 3’ Homologous free 
ends are required for stable joint molecule formation by the 
RecA and single-stranded binding proteins of Escherichia coli. 

Kornberg, A., 1980 DNA Replication. W. H. Freeman, San Fran-
cisco.

Kushner, S. R., H. Nagashii, A. TEMPLIN and A. J. Clark,
1971 Genetic recombination in Escherichia coli: The role of 

Lam, S. T., M. M. Stahl, K. D. McMillin and F. W. Stahl,
1974 Rec-mediated recombinational hotspot activity in bac-
teriophage lambda. II. A mutation which causes hotspot activ-

McEntee, K., 1977 Protein X is the product of the recA gene of 

56: 435–466.

Ponticelli, A. S., D. W. Schultz, A. F. Taylor and G. R. Smith,
1985 Chi-dependent DNA strand cleavage by RecBC enzyme.
Cell 41: 145–151.

Radicella, J. P., E. A. Clark, and M. S. Fox, 1988 Some 
Sci. USA 85: 9674–9678.

Rapoza, S. and M. S. Fox, 1987 Some features of base pair 
mismatch and heterology repair in Escherichia coli. Genetics

Rosenberg, S. M., 1987 Chi-stimulated patches are heteroduplex, 
with recombinant information on the phage λ λ chain. Cell 48:
855–865.

Rosenberg, S. M., 1988 Chain-bias of E. coli Rec-mediated λ 
patch recombinants is independent of the orientation of λ cos.
Genetics 119: 7–21.

Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill and G. B.
Petersen, 1982 Nucleotide sequence of bacteriophage λ 

Scheuermann, R. H., and H. Echols, 1984 A separate editing 
exonuclease for DNA replication: the ϵ subunit of Escherichia 
USA 81: 7747–7751.

Scheuermann, R. H., S. Tam, P. M. J. Burgers, C. Lu and H.
Echols, 1983 Identification of the ϵ subunit of Escherichia 
coli DNA polymerase III holoenzyme as the dnaQ gene product:
USA 80: 7085–7089.

Schultz, D. W., J. Swindle and G. R. Smith, 1981 Clustering 
of mutations inactivating a Chi recombinational hotspot. J.

coli RecBC pseudorevertants lacking Chi recombinational hot-

Shibata, T., C. DasGupta, R. P. Cunningham, and C. M. Rad-
ding, 1979 Purified Escherichia coli recA protein catalyzes 
homologous pairing of superhelical DNA and single-stranded 

Siegel, E. C., and J. J. Ivers, 1975 mut-25, a mutation to mutator 

Siegel, J., 1974 Extent and location of DNA synthesis associated 
with a class of Rec-mediated recombinants of bacteriophage 

Signer, E. R., and J. Weil, 1968 Recombination in bacteriophage 
Biol. 34: 261–271.

Smith, G. R., 1975 Deletion mutations of the immunity region of 

Smith, G. R., 1983 General recombination. pp. 175–209 in 
Lambda II, edited by R. W. Hendrix, J. W. Roberts, F. W.
Stahl and R. A. Weisberg. Cold Spring Harbor Laboratory, Cold 
Spring Harbor, N.Y.

Smith, G. R., 1987 Mechanism and control of homologous recom-

Smith, G. R., 1988b Homologous recombination in procaryotes. 

Smith, G. R., 1988b Homologous recombination sites and their 
recognition, pp. 115–154 in The Recombination of Genetic Ma-

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