RECOMBINATION PATHWAY SPECIFICITY OF CHI

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ABSTRACT

Chi in phage λ is a genetic element increasing the rate of recombination in its vicinity. Chi activity requires the wild-type functions of both the *recA* and the *recB* genes of *E. coli*. In terms of the pathway concept for recombination, Chi is active in the RecBC pathway and inactive in the Red, RecE, and RecF pathways.

GENETIC elements, called Chi, which act as stimulators of recombination by the Rec system of *E. coli*, arise by spontaneous mutation in at least four widely separated places in phage lambda (STAHL, CRASEMANN and STAHL 1975). They are identified as large-plaque mutants of *red⁻ gam⁻* phage, and their plaque morphology is causally related to their recombination-stimulation. Chi stimulates Break-Join exchanges (LAM *et al.* 1974), resulting in dimers when the participating chromosomes are monomeric circles. The dimers are deduced from the fact that Chi-stimulated exchanges promote chromosome encapsidation, for which a serially repetitious state of the lambda chromosome is prerequisite. Elements with properties identical to those of Chi have been identified in the wild-type *E. coli* chromosome (MCMILIN, STAHL and STAHL 1974; MALONE, in preparation) at a density of about one per ten genes.

In *gam⁻* infections, the *recBC* nuclease of *E. coli* remains active (UNGAR and CLARK 1972). Thus, in *red⁻ gam⁻* crosses, most lambda recombination occurs via the *E. coli* pathway normally operating during bacterial conjugation, *i.e.*, the RecBC pathway (GILLEN and CLARK 1974). Each Chi element in lambda stimulates crossing over maximally near itself but also as far away as half the length (about 20 genes) of the lambda chromosome (LAM *et al.* 1974; STAHL 1976). Thus (if Chi is active in the absence of lambda), the domains of the Chi elements in the *E. coli* chromosome are apt to be overlapping, and most of the recombination observed in bacterial conjugation or transduction may be Chi-stimulated.

Our view of bacterial recombination has been enlarged by A. J. CLARK's studies on *rec* mutants and systematized by the pathway concept to which these studies have lead (see CLARK 1974). In this contribution we have characterized Chi according to the pathways for which it stimulates recombination in lytically growing lambda. In addition to the RecBC pathway, we examine the RecE and

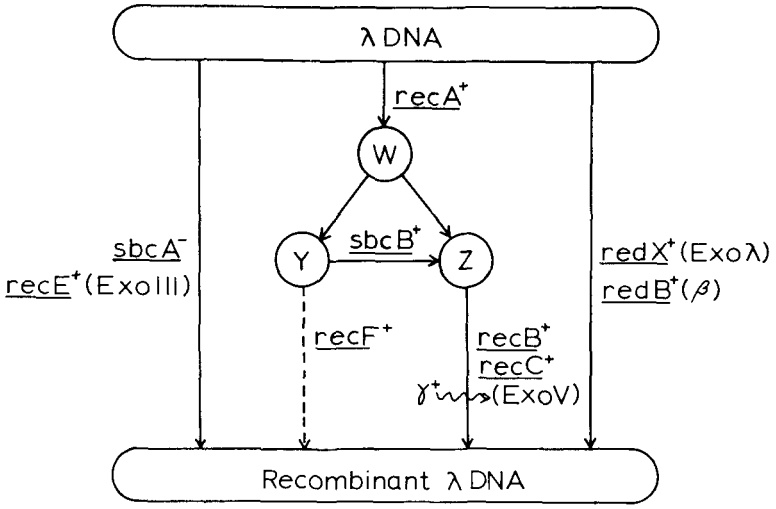

FIGURE 1.—Pathways of general recombination of lambda. This scheme by JANE GILLEN (1974) summarizes her experiments on the pathways of recombination for lambda. It is based on frequencies of recombinants (with a watchful eye on total yields) in crosses performed in the presence of active alleles of the various *E. coli* and lambda recombination genes. The dashed line from intermediate Y signifies that some recombinants go that route in wild-type crosses, more recombinants go that route in *gam*+ (*red*⁻ *sbcA*+) crosses, and in *recB*⁻/*C*⁻ (*red*⁻ *sbcA*+) crosses almost all recombinants go by that route. Our results mainly agree with GILLEN's and strengthen them, as described in the text. In only one respect did our experiments cast doubt on this scheme. We did find lower recombinant frequencies (with *red*⁻ phage) in the *recB recC sbcB recF* strain than in the *recB recC sbcB* one, but our "Int test" (see RESULTS) failed to show the anticipated large relative contribution of the Int system to that frequency (data not presented here). Perhaps *recF* is involved in Int-mediated recombination.

RecF pathways of *E. coli* and the Red pathway of lambda. The currently applicable formal scheme as it applies to lambda is shown in Figure 1.

Studies on the possible involvement of Chi in Red-mediated recombination have not been previously reported. On the other hand, GILLEN (1974) and GILLEN and CLARK (1974) concluded that *recA* function was required for Chi activity and that Chi played no role in recombination by the RecE or the RecF pathways. However, LAM *et al.* (1974) and HENDERSON and WEIL (1975) drew conflicting conclusions regarding the requirement for *recB* function in Chi activity. None of these studies was thoroughly convincing. The experiments of LAM *et al.*, and of GILLEN and CLARK were conducted in the presence of an active Int system, and one of the intervals used in assessing Chi activity included *att*, the site of Int exchange. The crosses of HENDERSON and WEIL involved parents with Chi's at different sites in the same cross. The Chi mutations did seem to alter the outcome of crosses in a *recB* host but the results were complex and not subject to straightforward interpretation.

We have constructed strains and devised procedures free of the shortcomings which characterized the previous studies. Our results show that *recBC* function is required for Chi to be expressed in recombination mediated by the Rec system.

When recombination occurs in the presence of Red, both *recA* and *recB* functions must be present for Chi activity.

Failure to see Chi activity in any particular host need not mean that Chi is inactive in the recombination pathway provided; it could mean that lambda (or lambda bearing Chi) is simply failing to use that pathway, recombining instead by some route not included in GILLEN's modification of CLARK's scheme (Figure 1). Therefore, we took pains to show that lambda (and lambda Chi) was using each provided pathway, and we describe those results herein.

MATERIALS AND METHODS

Bacterial strains: Strains used as hosts for crosses are tabulated in RESULTS. The REM strains are an isogenic set constructed by R. E. Malone and Jane Gillen for our Eugene collection. The JC strains which are Su+ were developed by A. J. Clark from AB1157. The JC strains which are Su⁻ are a second isogenic set derived by Clark. JC strains were obtained direct from Clark's lab, except where indicated.

Except when it was necessary to enumerate amber phage, platings were on strain 594, which gave plaques of convenient size and sharply discriminated between *cI* and *cI*+.

Genotypes of bacterial hosts were verified by us as follows: (1) *recB/C* was signaled by the plaque size made by the *red*⁻ *gam*⁻ deletion mutant *b*1453. Since the poor growth of *red*⁻ *gam*⁻ phage on wild type bacteria is due to interference by ExoV of phage DNA replication, good growth of such phage is diagnostic for *recB/C*. (2) *recB/C* was further verified by inability to produce lysogens when challenged with P2 phage. (3) Suppression of the *recB/C* phenotype (*i.e.*, the presence of *sbcA* or *sbcB*) was verified by experiments described in RESULTS. (4) *sbcA* was distinguished from *sbcB* by a relatively high eop for lambda particles not previously modified by the K restriction-modification system (SIMMON and LEDERBERG 1972).

Phage: The following mutants were used in constructing the phage genotypes employed:

As markers	*Reference*
*ts*A14	BROWN and ARBER (1964)
*ts*R2	BROWN and ARBER (1964)
*am*J6	WEIL and SIGNER (1968)
*am*R5	WEIL and SIGNER (1968)
*cI*26	MESELSON (1964)
Recombination-related	
*red*3	SIGNER and WEIL (1968)
*gam*210	ZISSLER, SIGNER and SCHAEFER (1971)
*int*4	GINGERY and ECHOLS (1967)
χA131	STAHL, CRASEMANN and STAHL (1975)
χB121	STAHL, CRASEMANN and STAHL (1975)
χD123	STAHL, CRASEMANN and STAHL (1975)
Δb1453	HENDERSON and WEIL (1975)

Cross procedures: (a) For measuring Chi activity and Int activity—type b (see RESULTS): A mixture of 7.5×10^8/ml of each of two genotypes was added to an equal volume of bacteria grown to 1.5×10^8/ml (except where noted) in broth with maltose (0.2%), thymine (20 μg/ml), and thiamine (20 μg/ml). The infected culture was rotated, shaken or bubbled at 37° for 30 minutes and then diluted 2000-fold and 40-fold further in prewarmed broth with additives. Both dilutions were incubated with aeration for an additional hour and then shaken with chloroform. Platings were made from the appropriate tube as determined by trial platings. The plates were incubated at 40° to examine recombinants in crosses with *ts* markers. In crosses involving *am* mutations, plates were incubated at 37°.

(b) For measuring recombination frequencies and Int activity—type a: Crosses were modi-

fied to include inactivation of unadsorbed phage by anti-lambda serum. Total phage progenies were then measured on plates incubated at 30°, and *ts*+ recombinants were assayed at 40°.

RESULTS

Response to Chi of four pathways and some combinations: Most of our crosses to measure Chi activity were of the following form:

tsA	x^+A		cI		+	
		×				Cross A
+	x^+A		+		tsR	
tsA			cI	x^+D	+	
		×				Cross D
+			+	x^+D	tsR	

where x^+ signifies an active (mutant) Chi site

In Cross A, the ratio of *ts*+ recombinants that are *c* to those that are c^+ is high when Chi is active. In Cross D, a low value of c/c^+ signals Chi activity. In the absence of Chi activity, the product (c/c^+ for Cross A) × (c^+/c for Cross D) should equal unity. Chi is active when this product significantly exceeds unity. We report the square root of the product as "Chi activity." Chi activity, so measured, is the geometric mean of the factor by which Chi in each of the two marked intervals alters the c/c^+ ratio. Thus, each reported activity summarizes the outcome of two experiments. By way of illustration, the first Chi activity reported in Table 1a has the value 4.6 which was arrived at as follows: In Cross A, the observed ratio of *c* to c^+ plaques was 906/80. In Cross D, the ratio of c^+ to *c* plaques was 171/91. The square root of the product is 4.6. Total phage yields in these experiments were not measured.

In order that the Int system operating on *att* in the interval A-*cI* not interfere with measurements of Chi activity, all phages were *int*⁻. All phages were *gam*⁻ so that crosses could be performed in which the *recBC* nuclease (ExoV) was not inhibited. The *gam* mutation used is an amber, and some of the bacterial strains are Su+. When the strain is also *recB/C*, the resulting suppression is expected to be inconsequential; when the strain is *rec*+, suppression will produce a partial *recBC* phenocopy. When it was desired to remove the Red system, phages carried *red3*.

Chi activities for the various crosses are reported in Table 1. The following points should be noted and conclusions drawn:

1. Any cross lacking *recA* or *recB* function shows no Chi activity. Therefore, both *recA* and *recB* functions appear to be needed for Chi activity.
   (a) If λ does use the RecE and RecF pathways, then Chi is not active in those pathways.
   (b) Red does not use Chi.
2. In *red*+ *rec*+ crosses intermediate Chi activity is observed. The presence

TABLE 1A

*Chi activity measured in the intervals A-*cI *and* cI-*R using* int*4*, red*3 *and* am gam*210 *to remove recombination functions*

Red system	Host	Host genotype	Chi activity	Avg.	Pathway(s)
—	594		Su⁻ 4.6		
—	594*		Su⁻ 4.7	4.9	Rec BC mostly
—	REM199		Su⁻ 5.3		
—	AB1157		Su⁺ 2.3	2.3	Rec BC reduced§
+	594		Su⁻ 3.1		
+	REM199		Su⁻ 3.3	3.2	Red and Rec BC
+	REM200	*recA recB*	Su⁻ 0.97		
+	REM201	*recA*	Su⁻ 1.2	1.1	Red
—	JC9387	*recB recC sbcB*	Su⁻ 0.96		
—	JC7623	*recB recC sbcB*	Su⁺ 1.0	1.0	Rec F
—	JC7623†	*recB recC sbcB*	Su⁺ 1.1		
—	REM202	*recB*	Su⁻ 1 2		
—	JC5519	*recB recC*	Su⁺ 1.1	1.1	Rec F low level
—	JC5519†	*recB recC*	Su⁺ 0.97		
+	REM202	*recB*	Su⁻ 0.87		
+	JC5519	*recB recC*	Su⁺ 1.1	0.99	Red and Rec F low level
—	JC9388‡	*recB recC sbcA*	1.1		
—	JC8679	*recB recC sbcA*	Su⁺ 1.1	1.1	Rec E
—	JC8679†	*recB recC sbcA*	Su⁺ 1.1		

* Saturated culture.
† From Dr. Jerry Cohen.
‡ This isolate obtained from John Clark was verified as recb/C sbcA but proved to be Su⁺ instead of Su⁻.
§ *gam*⁺ versions of these crosses were performed in AB1157, 594, and JC5519 giving Chi activities of 2.6, 2.0, and 1.0 respectively. Thus *gam* product cannot be counted on to produce a full *recB* phenocopy with respect to Chi activity.
¶ A value of unity implies that Chi does not act as a recombination hot spot.

of Chi activity in *red*⁺ *rec*⁺ crosses proves that its absence in *red*⁺ *rec*⁻ crosses is not due (entirely, at least) to inhibitory effects of Red function.

The experiments reported in Table 1 have a shortcoming. AB1157 is the parent strain to the Su⁺ series of *recB* derivatives. We presume our measure of Chi activity in AB1157 is diminished due to ExoV inactivation by *gam* product

TABLE 1B

*Chi activity measured in the intervals J-*cI *and* cI-*R using* Δ*b1453 to remove the* int, red, *and* gam *functions*

Host	Host genotype	Chi activity	Pathway
594		5.2	Rec BC mostly
AB1157		6.1	Rec BC mostly
JC7623	*recB recC sbcB*	1.2	Rec F
JC5519	*recB recC*	1.1	Rec F low level
JC8679	*recB recC sbcA*	1.2	Rec E

resulting from suppression of *am gam*210, and that presumption is supported by the outcome of *gam*$^+$ crosses (Footnote 4, Table 1). To gain further support for this presumption, we derived a second set of lambda strains with the help of R. E. Malone. These strains are *int*$^-$ *red*$^-$ *gam*$^-$ by virtue of the depletion *b*1453. The strains were marked as follows:

amJ	x^+B		cI		+
		×			Cross B
+	x^+B		+		amR
amJ			cI	x^+D	+
		×			Cross D′
+			+	x^+D	amR

Chi activities were determined from platings of the progenies on the Su$^-$ indicator 594 as the square root of the product (c/c^+ in Cross B) × (c^+/c in Cross D′). The results, Table 1b, show that AB1157 has as much Chi activity as does 594 when the infections are truly *gam*$^-$, and that results for the RecE and RecF pathways are as before.

Verification that RecE and RecF pathways act on lambda: GILLEN (1974) and GILLEN and CLARK (1974) provided evidence that the RecE and RecF pathways do, in fact, act on lambda by measuring recombinant frequencies in lysates from well-controlled crosses. One must have some reservations about the criterion, however. It is now apparent (STAHL *et al.* 1972a,b; ENQUIST and SKALKA 1973) that replication and recombination in lambda are, to a degree, interdependent, and that chromosome encapsidation is dependent on the prior occurrence of at least one of these processes. Thus, recombinant frequency among mature phage need not be a straightforward measure of recombination rate. For instance, an increased recombinant frequency due to an *sbc* mutation could be the result of either an increased recombination rate or an increase in the dependence of encapsidation on recombination as a result of a change in either the mode or amount of DNA replication.

A second method for assessing the amount of generalized recombination is to compare the outcome of *int*$^+$ and *int*$^-$ crosses. We expect the contribution due to Int to be minor in a bacterial host with a high rate of generalized recombination (a Rec$^+$ host) and major in a Rec$^-$ one (SIGNER and WEIL 1968). This technique has potential drawbacks, too. For instance, some of the genes involved in Rec recombination might influence Int recombination, as well. The weaknesses in this method appear different from those in the first one, however, so that concurrence of results by the two methods is good evidence for the activity or lack thereof of a particular Rec pathway on lambda.

We performed two sets of experiments.

(i) Crosses in the absence of Chi to measure recombinant frequencies and Int activities. The crosses were

tsA	att	cI	+
		×	
+	att	+	tsR

with both parents *red*$^-$. On each host, the cross was performed in both an *int*$^+$ and an *int*$^-$ version. Recombinant frequencies (Table 2) are measured and reported as the fraction of *ts*$^+$ progeny in the *int*$^-$ cross. Int activity (Table 2) is measured in two ways: (a) As the *ts*$^+$ frequency in the *int*$^+$ cross divided by that in the *int*$^-$ cross, and (b) among the *ts*$^+$ recombinants as *c*/*c*$^+$ for the *int*$^+$ cross times *c*$^+$/*c* for the *int*$^-$ cross. In Table 2, we see that the Rec$^+$ strains have high recombination frequencies and low Int activities while the Rec$^-$ ones give reverse results.

(ii) Crosses in the presence of Chi to measure Int activity. It was necessary to show that pathways confirmed to operate on lambda in test (i) above, indeed operated on lambda in the presence of Chi. Thus, for those bacterial strains which are blind to Chi, we measured Int activities on Chi-bearing phage to confirm that the active pathway was indeed operating. The crosses were

tsA	att	cI	χ^+D	+
		×		
+	att	+	χ^+D	tsR

with both parents carrying *red*$^-$ *gam*$^-$ point mutations. On each host, the cross was performed in both an *int*$^+$ and an *int*$^-$ version. (Data for the *int*$^-$ version are the ones from Cross D used to calculate Chi activity.) Int activity in these

TABLE 2

Recombinant frequencies and "Int activities" in some of the host strains

Host	Genotype	Recombinant frequency	Avg.	Int activity (a)	Avg.	Int activity (b)	Avg.	Pathway
REM202	*recB*	0.26*	0.63	9.2*	5.3	8.9*	6.2	Rec F low level
JC5519	*recB recC*	0.81		3.3		4.8		
JC9387	*recB recC sbcB*	2.0	2.2	1.6	1.9	1.7	1.8	Rec F
JC7623	*recB recC sbcB*	2.3		2.1		1.8		
JC9388†	*recB recC sbcA*	6.3	5.5	1.4	1.4	1.0	1.1	Rec E
JC8679	*recB recC sbcA*	4.6		1.3		1.1		
REM199		2.0	2.0	1.4	1.4	1.7	1.7	Rec BC mostly

* One experiment only.

† One experiment with the Su+ version of "9388" (see Table 1) and the other with the proper Su$^-$ version obtained from JOHN CLARK.

The crosses were *tsAcI* × *ts*R with both parents *red*3 *gam*210. Recombinant frequency is the frequency of *ts*$^+$ recombinants in an *int*$^-$ version of the cross. Int activity (a) is the ratio of the *ts*$^+$ frequency from an *int*$^+$ version of the cross to the *ts*$^+$ frequency from the *int*$^-$ version. Int activity (b) is determined from the ratios of *cI* to *cI*$^+$ among *ts*$^+$ in the *int*$^+$ and *int*$^-$ crosses as (*cI*/*cI*$^+$) (*int*$^+$) × (*cI*$^+$/*cI*) (*int*$^-$). All entries except where indicated are the averages of two experiments.

crosses was measured in only one way—among the ts^+ recombinants as (c/c^+ for the int^+ cross) × (c^+/c for the int^- cross). The Int activities are given in Table 3, which shows that these activities are high for the *recB/C* strains but low for those *recB/C* strains which are either *sbcA* or *sbcB*.

DISCUSSION

How can we best fit Chi into GILLEN's pathway scheme (Figure 1)? Obviously, there is no way that Chi can be, *sensu strictu*, an initiator in the scheme as drawn. If Chi stimulated the production of W, Chi would be found to act in both the RecBC and the RecF pathways. Rather than abandon the attractive possibility that Chi is an initiator sequence, let us redraw the scheme (Figure 2) to one that is an equally good summary of the facts presently available.

In the scheme of Figure 2, there are three places where Chi could conceivably act—in the formation of Z from lambda DNA (in which case it could be an initiator sequence), in the transformation of Z to recombinant DNA, or in the conversion of Y to Z. The last possibility is the easiest to argue against. To do so, we must consider the possible role of Chi in conjugational recombination, for which the RecF and RecBC pathways are related by HORII and CLARK (1973) as we have drawn them in Figure 2.

The primary argument for the relevance of Chi to *E. coli* recombination is the high density of Chi in the coli chromosome (MALONE, in preparation) and the specificity of Chi for the ordinary (RecBC) pathway of E. coli reported here. A secondary argument relates to the relative effects of a *recB/C* mutation on *E. coli* recombinant frequency as compared to that on lambda. In coli, a *recB/C* mutation reduces the recovery of recombinants 100-fold. In lambda, however, the reduction observed is about three-fold. This mysteriously large difference between the effect of *recB* mutations on lambda and *E. coli* can be under-

TABLE 3

Int activities-type b in the presence of Chi

Host	Genotype	Int activity	Avg.	Pathway
REM202	*recB*	21†	15	Rec F low level
JC5519	*recB recC*	8.3		
JC9387	*recB recC sbcB*	2.6		
JC7623	*recB recC sbcB*	2.3	2.7	Rec F
JC7623*	*recB recC sbcB*	3.3		
JC9388	*recB recC sbcA*	1.8		
JC8679	*recB recC sbcA*	2.0	1.8	Rec E
JC8679*	*recB recC sbcA*	1.7		
REM199		1.6	1.6	Rec BC mostly

* From JERRY COHEN.

† Only 18 c+ plaques were counted in this int^+ cross. More than 50 plaques were counted in each remaining class in this case and in every class of all the other cases.

The crosses were *amJcI* × *am*R with both parents *red*⁻ *gam*⁻ χ⁺D. Int activity type b is measured among am^+ recombinants as c/c+ for an int^+ cross times c+/c for the corresponding int^- one.

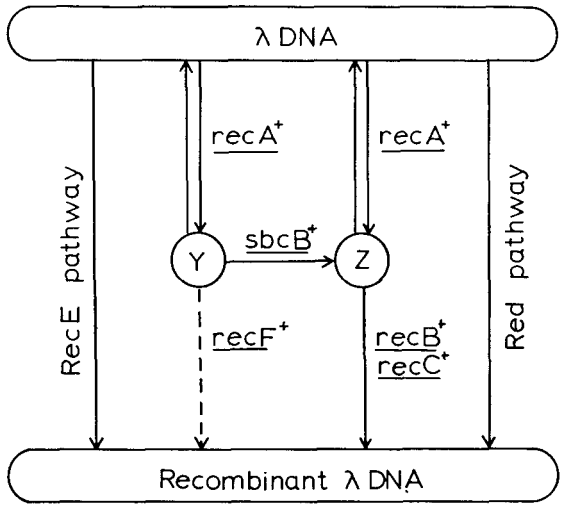

FIGURE 2.—Pathways for lambda recombination drawn to accommodate the possibility that Chi is an initiator sequence (after HORII and CLARK 1973).

stood, at least partly, from the simple fact that wild-type lambda has no Chi, combined with the simple assumption that Chi is active in *E. coli* conjugation. Thus the primary effect of eliminating *recBC* function is the elimination of Chi-recombination.

Is it quantitatively reasonable that Chi could be responsible for the approximately 30-fold difference in the responses of *E. coli* and lambda recombination to a *recB/C* mutation? The following calculation shows that it is. Chi sequences occur in wild type *E. coli* at a density of about one per ten genes (MALONE, in preparation). A single Chi sequence in lambda increases the total Rec-mediated recombination by a factor of five (MALONE and CHATTORAJ 1975), judging from the increase in burst size conferred by Chi. Since lambda is about 40 genes long, it would contain four Chi sequences if it contained them at the density that *E. coli* does. These four sequences would increase RecB-pathway recombination in lambda 20-fold over that found for lambda without Chi sequences. This factor of 20 is comparable to the factor of 30 we seek to explain, and we conclude that the primary effect of eliminating *recB/C* function in an *E. coli* conjugation may well be the elimination of Chi-recombination.

If Chi's position in the scheme were in the conversion of Y to Z, then elimination of that step by the mutation *scB* would seriously reduce recombination in an otherwise Rec$^+$ strain. It does not (HORII and CLARK 1973), and we conclude that Chi does not act there.

We see no way to choose between the remaining possibilities, but we can again draw on *E. coli* experiments, this time to define further the alternatives. According to BIRGE and LOW (1974), recombination in conjugation of *recB*$^-$ coli is blocked at a step after nearly normal levels of intragenic recombination have been achieved as measured by product produced by the recombinant gene. This observation allows us to say that if Chi is an initiator, its action is independent of

recBC, and we must postulate some other enzyme active on Chi. If, on the other hand, Chi acts below Z in the scheme, then ExoV itself may act on Chi.

A. J. CLARK was thoughtfully prompt in responding to our requests for strains and information via air to Jerusalem and Eugene. JERRY COHEN of Tel Aviv University supplied several "John Clark" strains when we became impatient with the post. We thank them, R. E. MALONE, and PENNY TOOTHMAN, who provided us with other strains.

The work reported confirms and extends an unpublished demonstration by ELLEN SINGER that Chi is not active in Red recombination.

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NOTE ADDED IN PROOF

The double mutant, *recFsbcB* (JC872), gives Chi activity indistinguishable from that of wild type. If Figure 2 is an adequate description of recombination pathways available to lambda, then all recombination of *int⁻red⁻gamma⁻* lambda in this host is down through intermediate Z. In that case, the lack of decrease in Chi activity supports the view that Chi does not act in the conversion of Y to Z. The lack of increase in Chi activity suggests that Chi acts in the conversion of Z to recombinant DNA.

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