REC-MEDIATED RECOMBINATIONAL HOT SPOT ACTIVITY IN BACTERIOPHAGE LAMBDA

II. A MUTATION WHICH CAUSES HOT SPOT ACTIVITY

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ABSTRACT

Crosses have been performed which identify phage mutants (*chi*) which cause recombinational hot spot activity in λ. The hot spot activity is found in crosses of *red*⁻ *gam*⁻ *chi*⁻ strains in *rec*⁺ hosts; in the crosses reported here, both the *chi*⁻ mutations and the hot spot are located near the right end of the chromosome. The hot spot occurs in standard crosses as well as under conditions which block DNA synthesis, and is dependent on a functional host *recB* gene. ——The *chi* mutation is shown to be dominant, but the tests do not show whether *chi* is a gene or a site.

GENETIC recombination in bacteriophage λ proceeds via three different systems; the host (*E. coli*) Rec system, and the phage Red and Int systems. Rec and Red are general recombination systems, while Int is site-specific (SIGNER 1971).

McMILIN and RUSSO (1972) developed a technique for conducting genetic crosses of λ in the almost complete absence of DNA duplication—a combined effect of mutations in the host *dnaB* gene and either the *O* or *P* gene of the phage. This technique of "double-block" crosses was used by McMILIN, STAHL and STAHL (1974) to survey for Rec-mediated recombinational hot spot activity in λ. To single out the Rec system, they used phage strains which had the entire λ recombination region deleted (Figure 1) and were phenotypically Spi⁻(LIN-

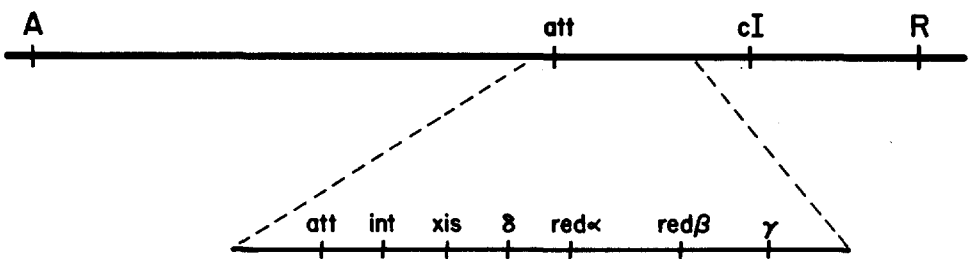

FIGURE 1.—Map of phage λ. Genes *int* through γ constitute the recombination region.

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DAHL *et al* 1970). Their results with *pure* deletion (as opposed to insertion–deletion) phage show recombinational hot spot activity located near the right end of the λ chromosome.

Several years ago, however, D. HENDERSON (personal communication) described a mutation which has the primary phenotype of conferring large plaque size on Spi⁻ deletion mutants. He observed that his Spi⁻ mutants were small plaque formers when first isolated, but that standard techniques inevitably resulted in stocks which were composed of large plaque forming phage—phage which carried HENDERSON's Spi⁻-associated mutation.

Thus, HENDERSON's mutation should have been present in the deletion strains which exhibited recombinational hot spot activity near the right end of the chromosome, and the present work confirms its occurrence in the *b1453* strains of MCMILLIN, STAHL and STAHL (1974). For reasons outlined in the DISCUSSION, we considered it likely that HENDERSON's mutation was in fact responsible for the recombinational hot spot activity. In the present paper we test this notion and further characterize the properties of HENDERSON's mutation.

Our first technical hurdle was to construct nearly isogenic phage strains, one set of which contained HENDERSON's mutation, the other set of which did not. This was facilitated by an observation which had earlier been made in this laboratory. JEAN CRASEMANN (personal communication) noted that, grown in an Su⁻ host, a *red3 gam210* point mutant would pick up a mutation—presumably HENDERSON's mutation—which converts the strain to a large plaque former. This led us to suspect that *red⁻ gam⁻* strains carrying HENDERSON's mutation would in addition exhibit recombinational hot spot activity. On the other hand, it is possible to maintain *red3 gam210* strains on a Su⁺ host and keep them free of HENDERSON's mutation (see STAHL *et al.* 1974).

In every experiment reported here, all of the phage strains carried the *red3 gam210* mutations—though the reader will not always be reminded of this fact. In the basic experiment, a pair of strains, each carrying HENDERSON's mutation, is crossed under conditions such that hot spot activity could be observed; simultaneously, a second pair of strains, each free of HENDERSON's mutation, is similarly crossed. The observation of hot spot activity involves a measurement of the fraction of J^+–R^+ recombinants occurring in the *cI–R* interval as compared with the fraction occurring in the *J–cI* interval. The latter interval includes *att*, the site of action of the Int system. Interpretation of some of the results must bear this in mind.

Following the Greek tradition of lambdologists, we shall hereafter refer to HENDERSON's mutation as *chi* (which can be thought of as the *c*rossover *h*ot-spot *i*nstigator). Thus wild-type phage are chi^+, and chi^- phage carry the mutation.

MATERIALS AND METHODS

Bacterial strains: The *Escherichia coli* strains used in this work are described in the companion papers or in Table 1. All *dnaB⁻* strains carry a temperature-sensitive mutation in the *dnaB* gene and were grown at 26°. In addition, T040 was grown in the dark.

Phage strains: The λ genotypes used in the crosses reported here are listed in Table 2. The identifying tests for the various mutations are described in STAHL *et al.* (1974) and MCMILLIN,

TABLE 1

Bacterial strains used and not described in companion papers

E. coli strain	Properties	Reference	Source
FA22	Su+, *recB*+, *dnaB*−	FANGMAN and NOVICK 1968	NOVICK
TO22	Su−, *recB*+, *dnaB*−	IRA HERSKOWITZ (personal communication)	IRA HERSKOWITZ
TO40	Su−, *recB*−, *dnaB*−	JEAN CRASEMANN and IRA HERSKOWITZ (personal communication)	JEAN CRASEMANN

STAHL and STAHL (1974). Phage carrying *red3 gam210* exhibit the Spi− and Fec− phenotypes in Su− hosts. The *chi41* and *chi22* mutations arose spontaneously and independently in the *b1453* strains of MCMILIN, STAHL and STAHL (1974).

The strains *tsA14 red3 gam210 cI26 amP80* and *red3 gam210 amP80 tsR2* were constructed by UV-stimulated crosses (Table 2, line 1). The construction of *chi*− analogs of these strains proceeded as follows: Strains *tsA14 b1453 cI26 amP80 chi41* and *b1453 amP80 tsR2 chi22* (from KEN MCMILIN) were crossed with *red3 gam210* phage which had been UV-irradiated. Unconditional Fec− recombinants (i.e., *b1453*) were selected against by plating on a Su+ *recA*− host, facilitating the recovery of the desired recombinants (Table 2, line 2). *P*+ derivatives of these *chi*− strains were obtained by picking revertants which plated on a Su− host (Table 2, line 3). *P*+ derivatives of the *chi*+ strains were constructed by recombination (Table 2, line 4).

In order to construct the strain *tsA14 red3 gam210 cI26 amP80 chi22* (Table 2, line 5), the strain *tsA14 red3 gam210 cI26 amP80 tsR2 chi22* was first constructed from a UV-stimulated cross. An *R*+ revertant of the double *ts* mutant was then selected by plating at 37°, taking advantage of the fact that *tsA14* plates at 37° (though not at 42°), while *tsR2* does not.

In order to construct the strain *red3 gam210 amP80 tsR2 chi41* (Table 2, line 5), the strains *tsA14 red3 gam210 amP80 tsR2 chi41* and *red3 gam210 cI26 amP80 chi41* were first constructed. The recombinant progeny from a UV-stimulated cross of these latter two strains yielded the desired strain.

The *chi*+ strains were at all times maintained on Su+ hosts. All phage stocks were made on C600, except for the density labeled stocks used in Figure 2, which were made by our standard techniques (see the companion papers).

Execution of experiments: Crosses in which DNA synthesis was blocked were performed as described in STAHL *et al.* (1974) and MCMILIN, STAHL and STAHL (1974) (see also MCMILIN and RUSSO 1972). Crosses in which DNA synthesis was not blocked differed in that a 10^4-fold dilution was carried out after the adsorption period. All crosses in temperature sensitive *dnaB*− hosts were carried out at 39°; in *dnaB*+ hosts at 37°. The lysates were plated on bacterial strain C600. The total phage platings were incubated at 30°; the *ts*+ recombinant phage platings at 42°.

TABLE 2

Phage genotypes used

1.	*tsA14 cI26 amP80*	*amP80 tsR2*
2.	*tsA14 cI26 amP80 chi41*	*amP80 tsR2 chi22*
3.	*tsA14 cI26 chi41*	*tsR2 chi22*
4.	*tsA14 cI26*	*tsR2*
5.	*tsA14 cI26 amP80 chi22*	*amP80 tsR2 chi41*

In addition to the indicated mutations, all of the phage strains listed here carried *red3 gam210*. The construction of these strains is described in the text in the same order that they are listed here.

RESULTS

The chi *mutation causes recombinational hot spot activity*

Our first task was to determine whether or not recombinational hot spot activity actually occurred with *red3 gam210 chi*$^-$ strains and if so, to determine the location of the hot spot. Figure 2 presents the results of a heavy-by-light cross designed to survey the chromosome between genes *A* and *R* for a possible recombinational hot spot; synthesis was blocked by the double-block technique. Density labeled *tsA cI chi41* was crossed with non-labeled *tsR chi22*. The skewed distribution of A^+R^+ recombinants indicates recombinational hot spot activity located near the right end of the chromosome, predominantly in the *cI-R* interval.

Is the *chi* mutation responsible for the recombinational hot spot activity? To answer this question, a pair of double-deck crosses were carried out in FA77, one cross being *chi*$^+$ (*tsA cI* × *tsR*), the other *chi*$^-$ (*tsA cI chi41* × *tsR chi*22).

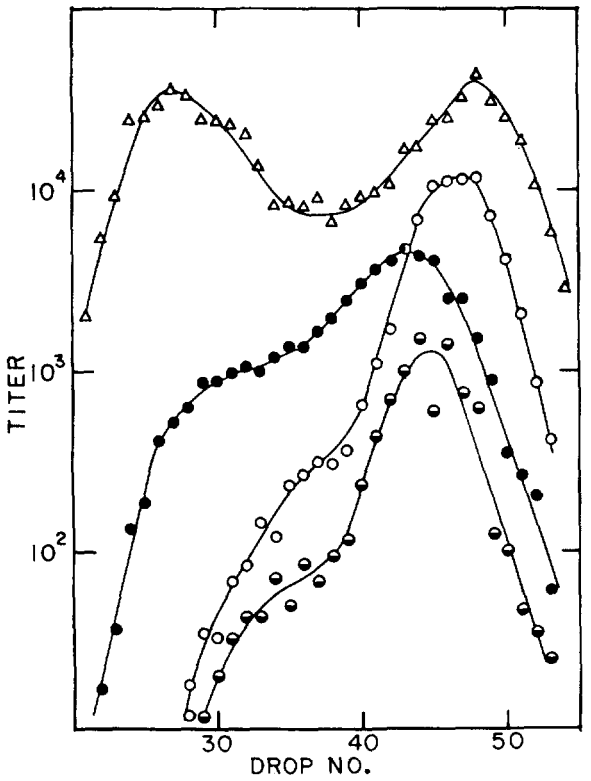

FIGURE 2.—Cesium density gradient of the lysate from a double-block cross in FA77 of *tsA cI chi41* × *tsR chi22*. The total phage (△) were assayed at 30°; *ts*+ phage at 42°. Clear *ts*+ phage (●) correspond to *A–cI* interval recombinants, turbid *ts*+ (○) correspond to *cI–R* interval recombinants, and mottled *ts*+ (◒) correspond to events at the *cI* locus. The *c–R/A–cI* ratio (ignoring mottled plaques) is 1.6. A *chi*+ experiment which is otherwise identical to the present one, is presented in Figure 5(a) of STAHL *et al.* (1974).

TABLE 3

Line number	Host	DNA synthesis	*cI–R/A–cI* ratio *chi*$^+$	*chi*$^-$
1	FA77	double block	0.26	4.8
2	594	*P*$^-$ block	0.22	3.8
3	594	no block	0.15	1.6
4	C600	no block	0.18	0.59
5	FA22	*dnaB*$^-$ block	0.39	1.3

All experiments were crosses of *tsA cI* × *tsR* in parallel with *tsA cI chi41* × *tsR chi22*, with one exception. In FA22 the *chi*$^-$ cross was *tsA cI chi22* × *tsR chi22*. In addition, the phage were always *red3 gam210* and occasionally *amP80*, as indicated in the text. Among the *ts*$^+$ recombinants in each lysate, the ratio of turbids to clears (*cI–R/A–cI* ratio) is taken as a measure of the *cI–R* interval recombinational hot spot activity. Please note, however, that this measure of the hot spot activity has a potential flaw. Above a certain level it might fail to reflect increases in the "absolute" level of the hot spot activity. This follows from the fact that the hot spot activity appears to extend to the left of the *cI* marker, so that, above a certain level, an increase in the level of the *cI–R* interval crossovers might be accompanied by a proportionate increase in the *A–cI* interval crossovers.

From each cross the resulting *ts*$^+$ recombinants were scored as clear (*cI*$^-$) and turbids (*cI*$^+$), corresponding, respectively to *A-cI* and *cI-R* interval crossovers. The ratio of turbids to clears (*cI–R/A–cI* ratio) is taken as a measure of the recombinational hot spot activity. The results (Table 3, line 1) indicate that the *chi* mutation is essential for this recombinational hot spot activity. Strictly speaking, the experiments presented here show only that the *chi* mutation or some other mutation inherited with it during the strain construction is responsible for hot spot activity. Experiments by JEAN CRASEMANN (manuscript in preparation), however, show that *chi*$^-$ mutations can occur at widely scattered points on the λ chromosome and that recombinational hot spot activity in their immediate vicinity is always observed. The ratio *cI–R/A–cI* of the physical lengths of the intervals is roughly 0.2 or 0.3; the *chi*$^+$ ratio is that expected for a uniform distribution of crossovers; the *chi*$^-$ ratio corresponds to roughly an 18-fold excess of *cI–R* interval crossovers.

Two crosses identical to those above were repeated, with the exception that the host was 594 and DNA synthesis was blocked solely by the λ *amP80* mutation. The results (Table 3, line 2) confirm that the *chi* mutation is essential for the recombinational hot spot activity.

Since it is known that under certain (*red*$^+$) conditions, the absence of DNA synthesis causes a non-uniform distribution of recombination events (STAHL *et al* 1972b), we wanted to know if the effect of the *chi* mutation would persist when extensive DNA synthesis is allowed. To answer this question, *P*$^+$ phage strains were constructed as described in MATERIALS AND METHODS. Crosses identical to those described above, but now using the *P*$^+$ strains, were carried out in 594. The data (Table 3, line 3) show nearly an 11-fold increase in the *chi*$^-$*cI–R/A–cI* ratio over the *chi*$^+$ ratio; the *chi* mutation apparently causes recombinational hot spot activity even in the presence of extensive DNA synthesis.

All of the above experiments are phenotypically *gam*$^-$. Since the *gam* gene appears to play a central role in both recombination and replication, we wanted

TABLE 4

Host	DNA synthesis	*recB*	*cI–R/A–cI* ratio *chi*+	*chi*-
TO22	double block	+	0.82	3.9
TO40	double block	—	0.26	0.33
JC4693	no block	+	0.16	1.8
JC4695	no block	—	0.13	0.18

All *chi*+ crosses were *tsA cI* × *tsR*. The double-block *chi*- crosses were *tsA cI chi22* × *tsR chi22*. The "no block" *chi*- crosses were *tsA cI chi41* × *tsR chi22*. In addition, the *red3 gam210* mutations were always present, and the *amP80* mutation was present in the double block crosses. Among the *ts*+ recombinants in each lysate, the ratio of turbids to clears (*cI–R/A–cI* ratio) is taken as a measure of the *cI–R* interval recombinational hot spot activity.

to know if the effect of the *chi* mutation would persist when the *gam* gene is expressed. Two variations on our basic experiment provide our answer to this question. In one, the host was C600, in the other, FA22. In C600 there was no block to DNA synthesis; in FA22, the *dnaB* block. In each case, the *gam210* mutation was suppressed, making the cross phenotypically *red*- *gam*+. (The *susP80* phage strains were used here, but this mutation too was suppressed.) The results (Table 3, lines 4 and 5) indicate that the presence of *gam* product tends to reduce the recombinational hot spot activity. Since *gam* product is known to inhibit the activity of the *recB–recC* DNase (SAKAKI *et al.* 1973) this result suggests a requirement for a functional *recB* gene in the hot spot activity.

To pursue this possibility, crosses were done in *recB*- hosts, with simultaneous *recB*+ control crosses. Two sets of experiments were performed, one set under double-block conditions, the other set under conditions where DNA synthesis was not blocked. The data (Table 4) show that similar results were obtained for both sets: the effect of *chi* on the ratio of recombinants was lost when the *recB* gene was not functional.

Crosses in which only one parent has the chi mutation

In order to test the *chi* mutation for dominance, crosses were carried out in which only one of the phage parent strains was *chi*-. The data are shown in Table 5. The fully *chi*- control is represented by the cross *tsA chi41* × *tsR chi22*. Two crosses of the type *tsA chi*+ × *tsR chi*- were carried out—one employing

TABLE 5

	tsA chi+	*tsA chi41*	*tsA chi22*
tsR chi+	0.12	0.72	0.69
tsR chi22	2.8	2.8	
tsR chi41	2.6		

All entries are from single-block (*P*-) crosses in 594 between *tsA cI* × *tsR*, with the presence or absence of either of the two independent *chi* mutations as indicated in the Table. In addition, all phage strains carried the mutations *red3 gam210 amP80*. Each entry in the Table is the ratio of turbids to clears (*c –R/A–cI* ratio) among the *ts*+ recombinants, which is taken as a measure of the *cI–R* interval recombinational hot spot activity.

chi22, the other *chi41*. In both cases the *cI–R/A–cI* ratio is essentially identical to the fully *chi*⁻ control. Similarly, two crosses of the type *tsA chi*⁻ × *tsR chi*⁺ were carried out—employing alternately *chi22* and *chi41*. Both of these crosses yield essentially the same *cI–R/A–cI* ratio, which is somewhat reduced from that of the fully *chi*⁻ control, but nevertheless quite distinct from that of the control where both parents were *chi*⁺. Taken all together, these results indicate that *chi*⁻ is dominant over *chi*⁺, and they suggest that there might be an asymmetry in the *chi*-instigated recombination process. That is, the apparent hot spot activity, judged from the *cI–R/A–cI* ratio, is greater if only the *tsR* parent is *chi*⁻, then it is if only the *tsA* parent is *chi*⁻, in crosses of the type *tsA cI* × *tsR*.

It was noticed when scoring the A^+R^+ recombinants, that plaques from the crosses *tsA* × *tsR*, *tsA* × *tsR chi22*, and *tsA* × *tsR chi41* were predominantly small, while those from the crosses *tsA chi41* × *tsR chi22*, *tsA chi41* × *tsR*, and *tsA chi22* × *tsR* were predominantly large. Presumably, a small plaque phenotype indicates a *chi*⁺ genotype, and a large plaque phenotype indicates a *chi*⁻ genotype. Therefore, almost all the crossovers that generated A^+R^+ recombinants occurred to the left of the *chi* mutation—be it *chi22* or *chi41*. Thus, both *chi22* and *chi41* map near the *R* gene. Deletion mapping has confirmed the location of these two *chi* mutations near *R* (J. CRASEMANN, personal communication).

DISCUSSION

In the accompanying paper (McMILIN, STAHL and STAHL (1974) it is shown that Spi⁻ deletion phage strains exhibit Rec-mediated recombinational hot spot activity near the right end of the chromosome. The present report shows that similar hot spot activity in *red*⁻ *gam*⁻ point mutant strains is caused by a mutation (HENDERSON's mutation) which we have named *chi*. By implication, the hot spot activity associated with the Spi⁻ deletions was probably caused by *chi* mutations.

We conclude that the hot spot activity is Rec-mediated because (1) the phage Red system is eliminated by mutation, and (2) the phage Int system acts only at the *att* site and thus could not contribute to *cI–R* interval crossovers. Furthermore, we have demonstrated that the hot spot activity is lost in the absence of a functional *recB* gene. This suggests that the effect of *chi* in producing recombinational hot spot activity is mediated through the *RecBC* pathway, as opposed to the possibility that *chi* activates some minor recombination pathway which is independent of the *recB* gene. While ruling out the notion that *chi*-stimulated recombination is independent of *recB*, our experiments fail to determine whether the residual *generalized* recombination in *recB*⁻ crosses (or *gam*⁺ crosses) involving *chi* is concentrated in the *cI–R* interval. Analagous crosses using phage which are additionally *int*⁻ are in progress to explore this question.

Crosses in which only one parent is *chi*⁻ show the *chi* mutation to be dominant, producing a relative excess of *cI–R* interval recombinants. However, it is not clear from the present data whether *chi* is a gene or a site. The *chi* mutations described here lie near the *R* gene.

Finally, we wish to consider what the role of the *chi* mutation might be in increasing the plaque size of *red*$^-$ *gam*$^-$ mutant strains. In particular, does the recombinational hot spot activity have anything to do with the plaque size increase? We believe the answer to be Yes. Growing in *RecA*$^-$ *recB*$^+$ hosts, *red*$^-$ *gam*$^-$ phage mature an abnormally small fraction of the DNA which they synthesize (ENQUIST and SKALKA 1973) and do not form plaques (ZISSLER, SIGNER and SCHAEFER 1971). Under these conditions the DNA is apparently trapped as a monomer, since both the recombination and rolling circle routes to multimer formation are blocked, and normal λ DNA cannot mature from a monomer (SZPIRER and BRACHET 1970; STAHL *et al.* 1972a; ENQUIST and SKALKA 1973; FREIFELDER, CHUD and LEVINE 1974; FEISS and MARQULIES 1973). In *recA*$^+$ *recB*$^+$ hosts, however, the Rec system apparently produces enough multimers that *red*$^-$ *gam*$^-$ (*chi*$^+$) phage grow and form plaques, but the plaques are typically very small. We suspect that the small size of these plaques is due to the low level of maturable, multimeric DNA produced by the Rec system under these conditions. In the presence of the *chi* mutation, however, the Rec system produces a greater level of maturable DNA. We imagine that either the absolute level of Rec recombination is greater under *chi*$^-$ as compared to *chi*$^+$ conditions, or else the products of *chi*$^-$ recombination are inherently more maturable than are the products of *chi*$^+$ recombination.

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