REC-MEDIATED RECOMBINATIONAL HOT SPOT ACTIVITY IN BACTERIOPHAGE LAMBDA. I. HOT SPOT ACTIVITY ASSOCIATED WITH SPI⁻ DELETIONS AND *bio* SUBSTITUTIONS

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ABSTRACT

In order to survey the distribution along the bacteriophage λ chromosome of Rec-mediated recombination events, crosses are performed using conditions which block essentially all DNA synthesis. One parent is density-labeled and carries a genetic marker in the left terminal λ gene (*A*), while the other parent is unlabeled and carries a genetic marker in the right terminal λ gene (*R*). Both parents are deleted for the λ recombination genes *int* and *red*, together with other recombination-associated genes, by virtue of either (1) a pure deletion or (2) a *bio* insertion-deletion. The distribution in a cesium density gradient of the resulting *A*+*R*+ recombinant phage reflects the chromosomal distribution of the recombination events which gave rise to those phage.

Crosses employing either of two different pure deletion phage strains exhibit recombinational hot spot activity located near the right end of the λ chromosome, between the *cI* and *R* genes. This hot spot activity persists when unlimited DNA synthesis is allowed. Crosses employing *bio1*-substituted phage strains exhibit recombinational hot spot activity located to the right of the middle of the chromosome and to the left of the *cI* gene. Crosses employing either *bio1* or *bio69*-substituted phage strains indicate that the *bio*-associated hot spot activity occurs in the presence of DNA synthesis, but is dependent on a functional host *recB* gene.

THE bacteriophage λ chromosome is subject to the action of three genetic recombination system: Int, which is site specific for the λ attachment locus, and Red and Rec, which are generalized (SINGER 1971). The products of the phage genes *int*, *redα* and *redβ* are essential components of their respective recombination systems (see Figure 1). The *redα* gene product—λ exonuclease—very likely is involved directly in the recombination process; the role of the *redβ* gene product—β protein—is unknown (RADDING 1973). Similarly, the *recB*–*recC* DNase of *E. coli* appears to be a central component of the Rec recombination system (CLARK 1973). However, residual recombination typically occurs in *recB*⁻ or *recC*⁻ hosts. By way of contrast, loss of the *rec*A gene product blocks all host-mediated recombination; the function of the *recA* gene is unknown.

The Red and Rec recombination systems presumably are capable of mediating recombination events essentially anywhere on the λ chromosome. Moreover, the

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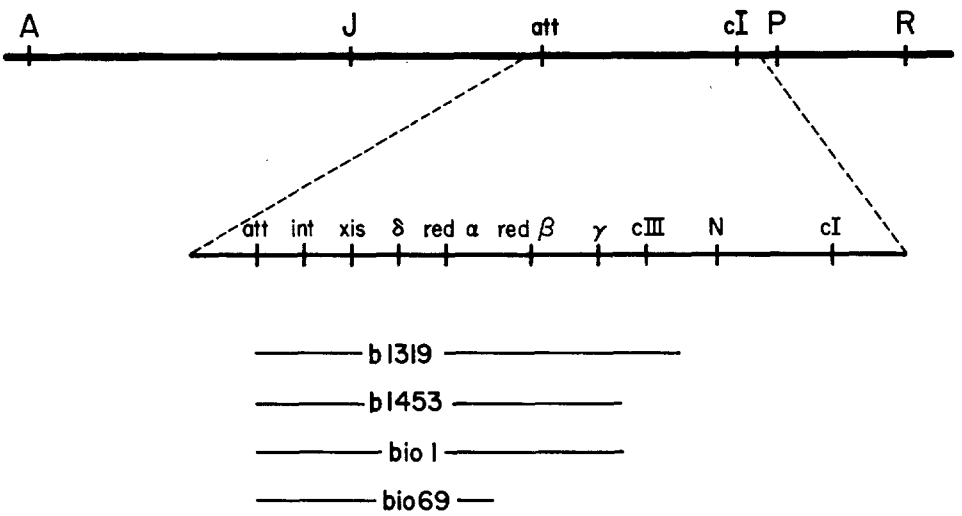

FIGURE 1.—Map of the λ chromosome indicating the genes relevant to this study. The expanded portion of the map includes the λ recombination region: genes *int* through *gam*. The portion of the λ chromosome deleted in phage carrying either *b1319*, *b1453*, *bio1* or *bio69* is indicated. The extent of the *bio* deletions is taken from the electron microscopy map of HRADECNA and SZYBALSKI (1969); the extent of the *b1319* and *b1453* deletions is determined from the following evidence: *b1319* and *b1453* are missing 12% and 11% of the λ DNA content, respectively, as judged from the intrinsic density of phage particles; both test as Spi⁻, Fec⁻, and *int*⁻; in addition, *1319* is apparently *cIII*⁻, since it makes only faintly turbid plaques and fails to complement *cIII67* (D. HENDERSON, personal communication; K. MCMILIN, unpublished results). Presumably phage which are Fec⁻, Spi⁻ by phenotype have as their genotype *δ*⁻, *red*⁻, *gam*⁻ (ZISSLER, SIGNER and SCHAEFER 1971a and b). Since frequently one end of a deletion is at the λ attachment locus (DAVIS and PARKINSON 1971), the present best guess for the extent of the *b1319* and *b1453* deletions is as indicated. Very recently, DNA heteroduplex mapping has confirmed that the left end of b1319 is at 0.572, the right end at 0.689 on the lambda scale of 0–1.0 (D. HENDERSON, personal communication). A portion of DNA from the biotin locus of *E. coli*, amounting to 5.2% of the wild type λ DNA content, is inserted at the locus of the deletion in *bio1* phage; the insertion is 8.5% in the case of *bio69* phage (HRADECNA and SZYBALSKI 1969). The evidence suggests that there is no insertion of *E. coli* DNA associated with the *b1319* and *b1453* mutations, but this suggestion has not been critically tested.

genetic linkage map determined from standard λ crosses is proportional to the physical map determined from the electron microscopy of heteroduplex DNA (CAMPBELL 1971), suggesting that generalized recombination acts uniformily along the length of the λ chromosome. Thus, it would seem that either there are no special sites on the chromosome (i.e., recombinational hot spots) at which generalized recombination preferentially occurs, or else there are very many (equally strong) recombinational hot spots distributed along the length of the chromosome. However, since λ recombination frequencies are normal under *red*⁺ *recA*⁻ conditions, and subnormal under *red*⁻ *recA*⁺ conditions (SIGNER and WEIL 1968), the Rec system is imagined to make only a minor contribution to the generalized recombinants in a standard λ cross; any possible hot spot activity

of the Rec system could be hidden by the uniform activity of the Red system. The present work is addressed to a search for Rec-mediated recombinational hot spots.

In addition to the phage genes *int* and *red*, other genes which cluster nearby on the λ map code for recombination-associated functions. In particular, the *gam* gene product is known to interact with the *recB–recC* DNase (SAKAKI *et al.* 1973) and, as a consequence, presumably modifies the host Rec recombination system. Thus, in order to study the unmodified Rec system, it is essential to eliminate the products of the *red* and *gam* genes, and it might be essential to eliminate the products of other recombination-associated genes—such as δ (J. ZISSLER, personal communication). In an attempt to eliminate all phage functions which might serve to obscure or modify the Rec recombination system, the present crosses are performed between phage deleted for all or most of the λ recombination region using either (1) pure deletion phage (D. HENDERSON, personal communication) or (2) *bio* insertion-deletion phage (HRAEECNA and SZYBALSKI 1969) (Figure 1).

In order to survey the entire λ chromosome in a single experiment for a possible recombinational hot spot, crosses are performed which in this manuscript are termed "hot spot survey crosses." These crosses are carried out using conditions which block nearly all DNA synthesis (MCMILIN and RUSSO 1972). Though there is some residual DNA synthesis, which is potentially very interesting (J. SIEGEL, manuscript in preparation), it is convenient and justifiable from internal evidence in the present experiments to imagine that absolutely all DNA synthesis is blocked (unless the contrary is specifically stated). One parent in each cross is density-labeled and carries a marker in the λ gene furthest left on the vegetative map (gene *A*), while the other parent is unlabeled and carries a marker in the furthest right λ gene (*R*). An A^+R^+ recombinant from this cross can conceivably be of essentially any density from nearly fully heavy to nearly fully light, depending on the locus of the recombination event. If there is a uniform distribution of recombination events along the chromosome, then it is reasonable to expect the distribution of A^+R^+ recombinants in a cesium density gradient to be flat (except, of course, at the ends of the distribution) as in Figure 2a. On the other hand, if at some locus in the chromosome there is a recombinational hot spot, then the A^+R^+ recombinants from events at the hot spot are expected to constitute essentially a single density species. Furthermore, if the strength of the hot spot is sufficiently great that most recombination events occur at the hot spot locus, then the density distribuion of A^+R^+ recombinants should have a rather sharp peak, as in Figure 2b.

For ease of presentation, it will be assumed that all recombination events are equally likely to lead to mature particle formation. In fact, however, the problem of selective maturation must ultimately be considered (STAHL *et al.* 1973).

This paper describes the existence of Rec hot spots in some λ strains. They are distinct from (and not to be confused with) the terminal "hot spotting" resulting from the action of the Red system on unreplicated phage described in the preceding companion paper.

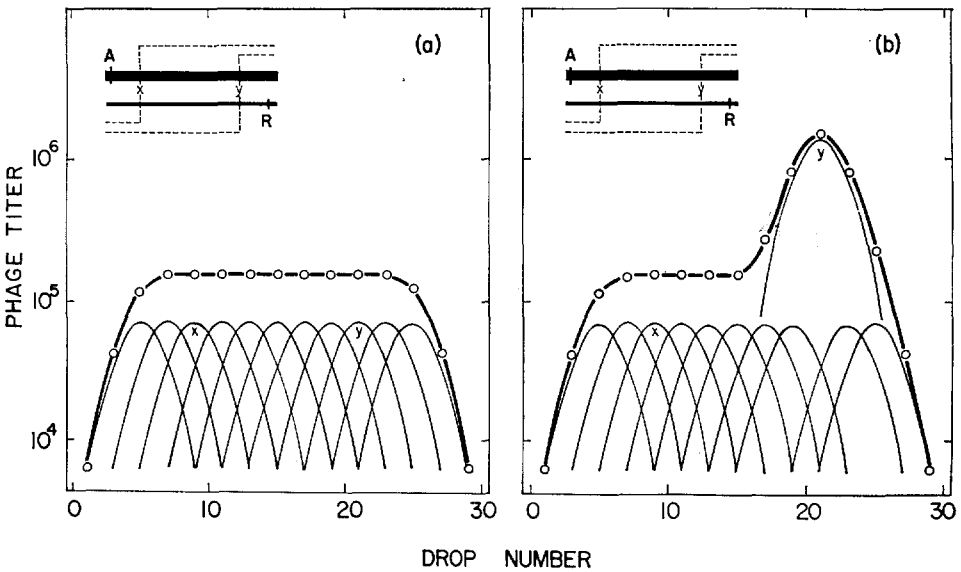

FIGURE 2.—The expected density distribution of $A+R+$ recombinant phage from hot spot survey crosses (○). In hot spot survey crosses the A^- parent is density-labeled heavy; the R^- parent is light; the cross is performed in the absence of DNA synthesis; and the resulting phage lysate is centrifuged to equilibrium in a cesium density gradient. The expected distribution of $A+R+$ recombinant phage, presented here, is calculated on the assumption that the product of a recombination event has a simple break and join structure with little or no hybrid overlap (MESELSON 1964). Thus, recombination events which occur at locus x, 20% of the chromosomal length from the left end of the molecule, yield $A+R+$ recombinants which are 20% light, 80% heavy, and band at the indicated locus in the gradient, centered at drop 9. (The phage titer and drop number scales are arbitrary. The distribution of phage corresponding to a single density species is taken from a typical gradient curve.) The complete distribution of $A+R+$ recombinants is found by adding the contributions from events located (arbitrarily) at 10% intervals along the entire length of the chromosome. Panel (a) shows the expected results corresponding to a uniform distribution of recombination events along the chromosome. On the other hand, a recombinational hot spot at locus y (of arbitrary magnitude) yields the expected density distribution of $A+R+$ recombinants presented in panel (b). Please note that these expected distributions must be viewed with some caution, because (1) we present no evidence in support of the break-and-join structure, and (2) the assumption of no hybrid overlap is clearly artificial; in fact, a fairly extensive hybrid overlap structure might by itself lead to a distribution which is humped in the middle, like that in Figure 5 of STAHL *et al.* (1974). Therefore, in so far as possible, the interpretation of the actual experimental results makes no *a priori* assumption as to the structure of a recombinant molecule.

MATERIALS AND METHODS

The λ mutations employed are the following: *tsA14*, *tsJ15*, *tsR2* from W. ARBER (BROWN and ARBER 1964); *tsR3* from L. SIMINOVITCH (HARRIS *et al.* 1967); *cI26*, *amP80* from M. MESELSON (MESELSON 1964; CAMBELL 1961); *gam210* from E. SIGNER (ZISSLER, SIGNER and SCHAEFER 1971a); *b1319*, *b1453*, *bio69*, *cI857* from D. HENDERSON; *bio1* from N. MURRAY; for the *bio* substitutions, refer to HRADECNA and SZYBALSKI (1969). Derivative phage strains, constructed from the above mutants, are presented at the appropriate place in the text.

A number of tests serve to verify that the derivative strains carry the desired mutations. phage carrying *amP80* fail to plate on a Su^- host. Phage carrying a ts^- marker fail to plate

at 42°; moreover, phage carrying the *tsR2* marker fail to plate at 37°, distinguishing *tsR2* from the other *ts*⁻ markers used in this study. The various *ts*⁻ markers are further distinguished and verified by virtue of their ability or inability to complement and recombine with the *ts*⁻ markers in the primary strains. Phage carrying *b1319*, *b1453*, *bio1*, and *bio69 gam210* exhibit the Spi⁻ and Fec⁻ phenotypes. Spi⁻ strains plate on P2 lysogens; Fec⁻ strains fail to plate on *recA*⁻ hosts (ZISSLER, SIGNER and SCHAEFER 1971a and b). In addition, the intrinsic densities of the primary strains carrying *b1319*, *b1453*, *bio1* and *bio69* differ substantially from each other and from wild-type λ. For some of the derivative strains the intrinsic density differs very slightly, for unknown reason, from that of the corresponding primary strain. All of the *bio1*-substituted derivative strains are specifically known to be *int*⁻ and *red*⁻ as verified by their inability to recombine with other *int*⁻ *red*⁻ strains in a *recA*⁻ host. Additional evidence that the derivative strains are correctly constructed is implicit in the data presented in RESULTS.

Values cited in the text for the ratios of the physical lengths of various intervals on the λ chromosome are determined with the aid of the map of SZYBALSKI (1971).

Strains carrying the *b1319* and *b1453* deletions typically also carry an associated mutation (named *chi*) which is separable from the deletion and confers increased plaque size on the deletion strain (D. HENDERSON, S. LAM, J. CRASEMANN and M. STAHL, personal communications). The *b1453* strains (and presumably also the *b1319* strains) used in the present work carry associated *chi* mutations (S. LAM and M. STAHL, personal communication). The *chi* mutation is implicated in the recombinational hot spot phenomenon, as is discussed in the companion publication (LAM *et al.* 1974).

We do not know if the *bio1* or *bio69* substitution strains carry associated mutations (comparable to *chi*) which are separable from the substitution.

Table 1 lists the *Escherichia coli* host strains and their relevant properties. Strains carrying the *recB21* mutation have been verified to be UV sensitive relative to *recB*+ strains. C600 is used to score the progeny phage from all recombination experiments.

In all crosses, the multiplicity of infection for each parental strain is between 5 and 10 phage per cell. λ DNA synthesis is blocked in all hot spot survey crosses by the combination of the temperature sensitive *dnaB* mutation in the host FA77 and the λ *amP80* mutation. In the rest of this manuscript, the term hot spot survey cross implies that the host is FA77 and that both parental phage strains carry the *amP80* mutation. Some of the *recB*⁻ crosses (together with *recB*+ control crosses) presented in Table 2 are carried out in the dark. However, identical results are obtained when such crosses are carried out in the light.

The portion of each density gradient presented here includes only phage with light protein coats. Thus, relative positions in the density gradient correspond solely to the amounts of density label in the DNA of phage particles. The terms "fully heavy" and "fully light" refer to the DNA content of progeny phage particles, all of which in fact have light protein coats.

Other experimental details have been discussed previously (MCMILIN and RUSSO 1972).

TABLE 1

E. coli strain	Relevant properties	Reference	Source
FA77	Su⁻, *recB*+, *dnaB*⁻	MCMILIN and RUSSO 1972	——
594	Su⁻, *recB*+	WEIGLE 1966	J. PARKINSON
JC4693	Su⁻, *recB*+	KUSHNER *et al.* 1971	A. J. CLARK
W3102	Su⁻, *recB*+	ECHOLS and GREEN 1971	M. MESELSON
C600	Su+, *recB*+	APPLEYARD 1954	M. MESELSON
K12SH-28	Su+, *recB*+	FANGMAN and NOVICK 1966	A. NOVICK
AB1157	Su+, *recB*+	KUSHNER *et al.* 1971	A. J. CLARK
JC4695	Su⁻, *recB21*	——	A. J. CLARK
JC7720	Su+, *recB21*	——	A. J. CLARK
JC6722	Su+, *recB21*	——	A. J. CLARK
JC5519	Su+, *recB21*, *recC22*	WILLETS and CLARK 1969	A. J. CLARK

RESULTS

1. Hot Spot Survey Crosses

Figure 3 presents the results of a hot spot survey cross in which both parental strains carry the *b1319* deletion. As expected, there are A^+R^+ recombinants at all densities from nearly fully heavy to nearly fully light. The striking observation is that most of the A^+R^+ recombinants occur in a peak corresponding to nearly fully light phage particles. Since very frequently a combination of R^+ information from the heavy phage parent is associated with a small material contribution of DNA, the recombination event which mediates this result necessarily occurs near the right end of the chromosome, indicating that there is a recombinational hot spot near the right end of the chromosome.

Figure 4 presents the results of a hot spot survey cross in which both parental strains carry the *b1453* deletion. In addition, the chromosome is genetically dissected by virtue of a plaque morphology mutation in the *cI* gene. In Figure 4a the distribution of A^+R^+ recombinants peaks at the density corresponding to nearly fully light phage, indicating as before that there is a recombination hot spot near the right end of the chromosome. Genetic data available here, but unavailable in the experiment present in Figure 3, confirm and extend this observation. Among the A^+R^+ recombinants, the ratio of recombination events in the *A–cI* interval *vs.* the *cI–R* interval is 0.24; whereas, the ratio of the physical lengths of these intervals is about 3 or 4. Thus, the genetic data indicate a recombinational hot spot, located in the *cI–R* interval, such that the relative fre-

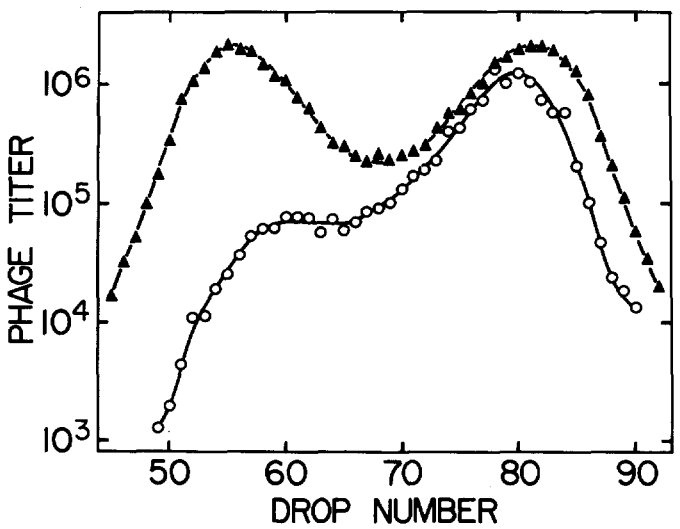

FIGURE 3.—The distribution in a cesium density gradient of the lysate from a hot spot survey cross between heavy *tsA14 b1319* and light *b1319 tsR2*. The overall phage (▲) are assayed at 30°; the A^+R^+ recombinants (○) at 42°. Fully heavy phage band at drop 55, fully light phage at drop 83. Thus, the peak of A^+R^+ recombinants, centered at drop 80, corresponds to phage which are roughly 10% heavy, 90% light.

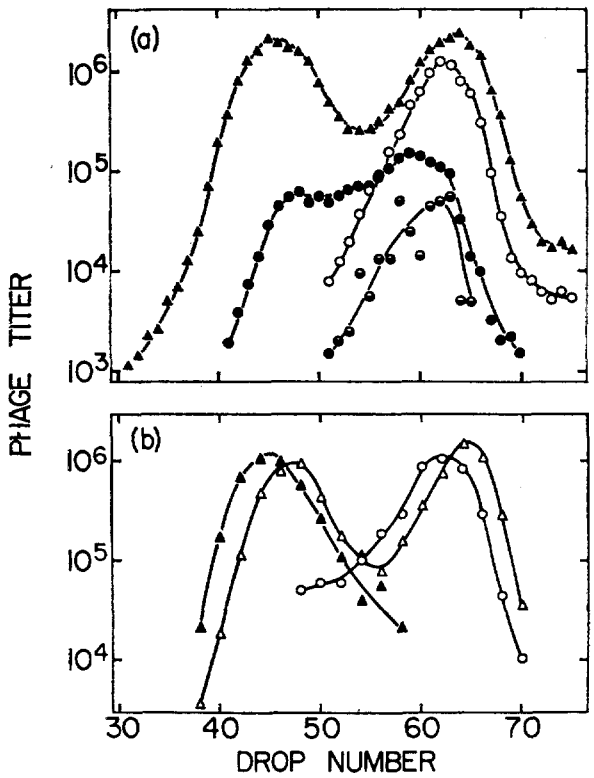

FIGURE 4.—The distribution in a cesium density gradient of the lysate from a hot spot survey cross between heavy *tsA14 b1453 cI26* and light *b1453 tsR2*.

(a) The overall phage (▲) are assayed at 30°. The A^+R^+ recombinants, assayed at 42°, are scored as clear (cI^-), turbid (cI^+), or mottled (cI^-/cI^+) plaques, corresponding, respectively, to events in the *A-cI* interval (●), the *cI–R* interval (○), or at the *cI* locus (◒).

(b) Every second tube in the gradient is plated for a small number of plaques (about 20 on the average) per plate at 30°. All well-isolated plaques (separated by at least 0.5 cm from the nearest neighbor) are picked and scored for genotype; roughly one quarter of the plaques are separated by less than 0.5 cm and are not scored. Both the *tsA14* and the *tsR2* mutations confer the inability to grow at 42°, but only the *tsR2* mutation confers the inability to grow at 37° Thus a simple spot test suffices to determine in large part the genotype for the *A* and *R* loci. according to the following rules. Phage which grow at 30°, 37°, and 42° are A^+R^+ (○). Phage which grow at 30° and 37°, but not at 42°, are A^-R^+ (▲). Phage which grow at 30°, but not at 37° and 42°, are either A^+R^- or A^-R^- (△). Among phage in this last class (A^+R^- or A^-R^-), 34 out of 34 at drop 48 are in fact A^-R^- and 41 out of 43 at drop 64 are in fact A^+R^-, as determined by spot complementation tests against known A^- and R^- strains. At drops 44 and 46, 99% of the A^-R^+ phage are clear (cI^-); at drops 60, 62 and 64, 91% of the A^+R^+ phage are turbid (cI^+). A^-R^+ parental phage band at drop 45, A^-R^- recombinant phage at drop 47 or 48, A^+R^+ recombinant phage at drop 62, and A^+R^- parental phage at drop 64 or 65. Since the A^-R^+ and the A^+R^- parental phage are fully heavy and fully light, respectively, the A^-R^- and the A^+R^+ recombinant phage are 85 to 90% heavy, in the one case, and 85 to 90% light, in the other.

quency of recombination events in the *cI–R* interval exceeds by a factor of 14 or 15 what would be expected in the absence of the hot spot activity.

Consistent with the current notion that λ DNA must be bounded by two *cos* sites in order to be maturable (ENQUIST and SKALKA, 1973; STAHL *et al* 1973; FEISS and MARGUILES 1973), it appears to be well established that recombination is essential for the maturation of unduplicated λ DNA (SZPIRER and BRACHET 1970; STAHL *et al.* 1972a; FREIFELDER, CHUD and LEVINE 1974). Thus all of the phage in the present experiment should be recombinant. Moreover, the majority of the overall phage should be recombinant in the *cI–R* interval. Since "mating" supposedly occurs at random with respect to genotype within a population of vegetative phage, it is expected that the overall phage should divide equally into the following four classes: (1) fully heavy $A^-cI^-R^+$ phage (the product of heavy by heavy hot spot recombination events), (2) nearly fully heavy $A^-cI^-R^-$ phage (one of the two products of heavy by light hot spot events), (3) nearly fully light $A^+cI^+R^+$ phage (the other product of heavy by light hot spot events), and (4) fully light $A^+cI^+R^-$ phage (the product of light by light hot spot events).

The data of Figure 4b provide virtually total confirmation that the overall phage divide equally into the expected four classes, confirming also the hypothesis that Rec-mediated recombination is capable of facilitating the maturation of unduplicated λ DNA. The class of A^-R^- recombinants is especially significant, for the following reasons. It is a fundamental assumption in this work that the relative density of a heavy by light recombinant reflects the relative contributions of DNA of each of the parental phage to the recombinant phage—as opposed to other events which would have a substantial effect on phage density, such as extensive DNA synthesis or the formation of sizeable deletions. If the above assumption is correct, then the reciprocal A^-R^- recombinants should peak at a density corresponding to nearly fully heavy phage. The fact that they do provides substantial confirmation of the interpretation that there is a recombinational hot spot near the right end of the chromosome. There is a similar class of nearly fully heavy A^-R^- recombinants in the yield of phage presented in Figure 3, further confirming the hot spot interpretation (unpublished results).

Figure 5 presents the results of a hot spot survey cross in which both parental strains carry the *bio1* substitution. The relatively narrow peak of $A^+cI^-R^+$ recombinants in panel (c) (to be contrasted with the broad, flat distribution of A^+R^+ recombinants in Figure 2a) indicates that under these conditions there is a recombinational hot spot (or spots) located somewhere between the middle of the chromosome and the *cI* gene. The requirement for recombination to facilitate maturation predicts that the overall phage should divide equally into four classes: (1) fully heavy $A^-cI^-R^+$ phage, (2) predominantly heavy $A^-cI^+R^-$ phage, (3) predominantly light $A^+cI^-R^+$ phage, and (4) fully light $A^+cI^+R^-$ phage. The data of Figure 5 imply that peak A consists of $A^-cI^-R^+$ phage; peak B consists of $A^?cI^?R^-$ phage; peak C consists of $A^+cI^-R^+$ phage; and peak D consists of $A^?cI^?R^-$ phage. Scoring the *A* and *cI* markers in the presence of the R^- marker

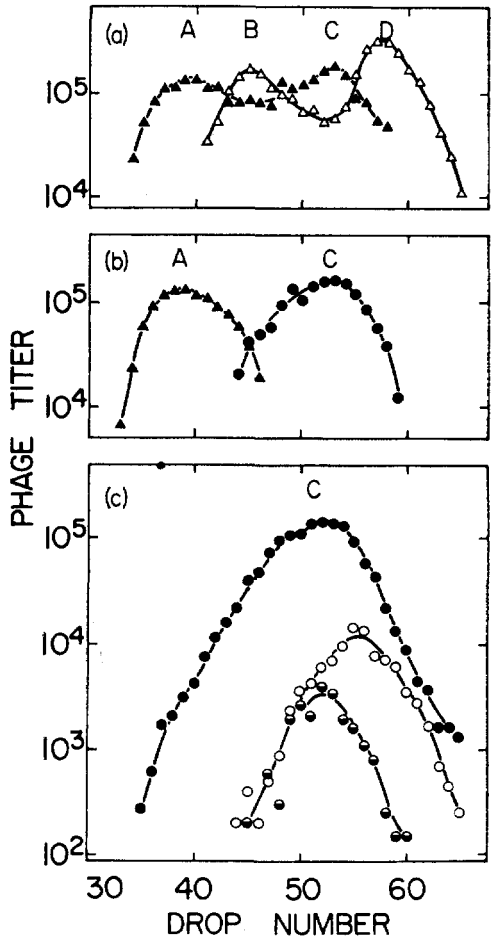

FIGURE 5.—The distribution in a cesium density gradient of the lysate from a hot spot survey cross between heavy *tsA14 bio1 cI26* and light *bio1 tsR2*.

(a) Overall phage, assayed at 30°, are divided into two classes: Medium size plaque formers (▲) and small plaque formers (△). The phage distribute into four distinct peaks: A,B,C,D. Since the *tsR2* mutation confers a small plaque phenotype, it is tentatively assumed that peaks A and C consist of $R+$ phage, peaks B and D, R^- phage.

(b) R^- phage are excluded by assaying at 34°, a non-permissive temperature for the *tsR2* mutation. The loss of peaks B and D confirms that they consist of phage carrying the *tsR2* mutation. The $R+$ phage are now distinguishable as medium size plaque formers (▲) and large plaque formers (●), which segregate into peaks A and C, respectively. Since the *tsA14* mutation confers reduced plaque size, it is tentatively assumed that peak A consists of A^-R+ phage, peak C of $A+R+$ phage. The vast majority of the phage in peak A form clear plaques (cI^-).

c) $A+R+$ phage, assayed at 42°, constitute peak C as expected, confirming the A^-R+ character of the phage in peak A. The relatively narrow, peaked distribution of the $A+R+$ phage at a density of less than half heavy, indicates a recombinational hot spot, located to the right of the middle of the chromosome. The major portion of $A+R+$ recombinants score clear (cI^-) (●), minor portions scoring turbid ($cI+$) (○) and mottled ($cI^-/cI+$) (◒), indicating that the recombinational hot spot is left of the *cI26* marker.

requires further analysis, which has not been carried out. Nevertheless, the data of Figure 5 suggests a *bio1*-associated, Rec-mediated recombinational hot spot, located between the middle of the chromosome and the *cI* gene, and add further confirmation that the Rec system is able to mediate recombination events that facilitate the maturation of unduplicated λ DNA.

In contrast to the distribution of Figure 2a, the A^+R^+ recombinants of Figure 5c are properly described as banding in a relatively narrow peak. However, compared to the distribution of phage in peak D, which ought to constitute a single density species, the A^+R^+ recombinants of peak C appear to band in a relatively broad peak. Indeed, the full width at half maximum of peak C is 8.5 drops; whereas, the same measurement of peak D is 5 drops. Two obvious explanations readily suggest themselves. Either the precise locus of the hot spot recombination event is somewhat variable, or there are several hot spots, or else a small, variable amount of DNA synthesis is associated with the event. Though the former possibility is not ruled out, the latter possibility is supported by the fact that the distribution of heavy by heavy recombinants in peak A is relatively broad. The full width at half maximum of peak A is 8 drops.

2. Crosses in which DNA synthesis is not blocked

It has previously been observed with *red*$^+$ crosses that the presence or absence of DNA synthesis can affect the distribution of recombination events along the chromosome (STAHL *et al.* 1972b and 1974). Thus it is imperative to test if the present nonuniform chromosomal distributions of Rec-mediated recombination events, observed in the absence of DNA synthesis, persist in the presence of DNA synthesis.

Figure 6 presents the results of a freely duplicating density transfer cross in which both parental strains are density-labeled heavy and carry the *b1453* deletion. The host strain AB1157 is light. DNA synthesis is monitored by the shift in density consequent to the appearance of light material in the progeny phage. Most of the phage in the yield are fully light and have necessarily duplicated at least twice. Nevertheless, the ratio of *A–cI/cI–R* recombinants is 0.29, in stark contrast to the ratio of the physical lengths of the intervals (about 3 or 4). Identical free duplication crosses conducted in other hosts, though without the monitor of density label, confirm the recombinational hot spot. The ratio of *A–cI/*cI–R recombinants is 0.50 from a cross in C600, and 0.41 from a cross in K12SH-28. Thus, the relative frequency of recombination events in the *cI–R* interval exceeds what would be expected in the absence of the hot spot activity by a factor of anywhere from 6 to 14.

It is somewhat more difficult to rule out the possibility that the absence of DNA synthesis is a prerequisite to the nonuniform distribution of recombination events associated with *bio1*-substituted phage. The problem is that the ratio of *A–cI/cI–R* recombinants in the hot spot survey cross of Figure 5 is 15, which is different, but not dramatically different, from the ratio of the physical lengths of the intervals. In order to confirm the recombinational hot spot by using the relative physical length of the interval as the standard of comparison, it is necessary to bracket the locus of the hot spot with markers that are substantially closer

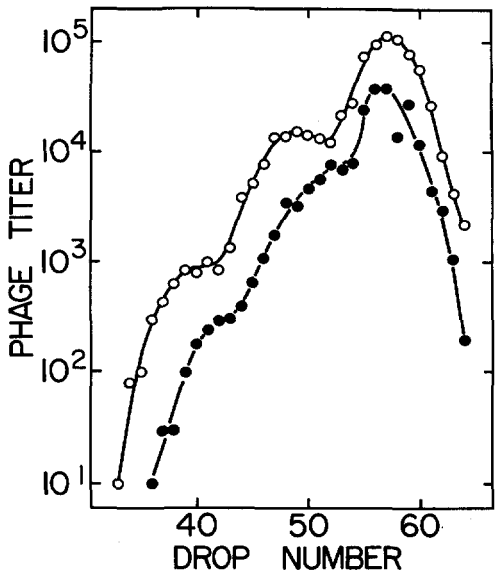

FIGURE 6.—Density-transfer cross between *tsA14 b1453 cI26* and *b1453 tsR2* in which both phage strains are density-labeled heavy and the cross is conducted in light medium in host strain AB1157. Both phage strains also happen to carry the *amP80* mutation, but it is suppressed in this host; the phage are freely replicating. The fully heavy, half heavy, and fully light regions of the gradient, centered roughly at drop 40, drop 49, and drop 57, consist, respectively, of unduplicated phage, phage which have duplicated at least once, and phage which have duplicated at least twice. Only the A^+R^+ recombinants are shown, and they are distinguished, by virtue of the *cI* mutation, as corresponding to events in either the *A–cI* interval (●) or the *cI–R* interval (○). The frequency of A^+R^+ recombinants is 7.2%. Unadsorbed phage band at drop 8.

together than those in genes *A* and *cI*. This has been attempted with markers in genes *J* and *cI*, and the results are presented below.

An additional approach is to seek non-hot spot recombination conditions to serve as the standard of comparison. This has more or less been achieved by performing crosses in $recB^-$ hosts. The rationale is that the residual recombination pathway exposed in a $recB^-$ host is expected to have some fundamentally different properties from the major pathway of $recB^+$ hosts. In particular, the two pathways might differ with respect to their recombinational hot spot behavior.

Table 2 presents the results for the ratio of recombinants in the *J–cI vs. cI–R* intervals from crosses of *bio*-substituted strains in a number of different $recB^+$ and $recB^-$ hosts. The ratios are comparable to one another in all $recB^+$ crosses (including one cross in which DNA synthesis is blocked), and they are comparable to one another in all $recB^-$ crosses. Since two different *bio* substitutions are used, this indicates that the *bio*-associated hot spot phenomenon is of some generality. But the main point is that the *J–cI/cI–R* ratios are dramatically different in the $recB^+$ compared to the $recB^-$ crosses. Moreover, it is the $recB^-$ crosses which are in good agreement with the *J–cI/cI–R* ratio from double-point mutant int^-

TABLE 2

Phage carrying	Host	DNA synthesis	*recB*	$\frac{J\text{–}cI}{cI\text{–}R}$
bio1	FA77	—	+	8.9
bio1	C600	+	+	5.9
bio1	K12SH-28	+	+	5.7
bio1	AB1157	+	+	7.6
bio1	JC7720	+	—	0.86
bio1	JC6722	+	—	0.74
bio69 gam210	594	+	+	12.
bio69 gam210	JC4693	+	+	8.3
bio69 gam210	28	+	+	11.
bio69 gam210	JC4695	+	—	1.1
bio69 gam210	JC7720	+	—	1.1
bio69 gam210	JC6722	+	—	1.4
bio69 gam210	JC5519	+	—	1.1

Crosses either of *tsJ15 bio1 cI26 amP80* by *bio1 amP80 tsR2* or of *tsJ15 bio69 gam210 cI857* by *bio69 gam210 tsR3*. The presence of DNA synthesis in all of the hosts except FA77 is inferred from the ability of the parental strains to form plaques on those hosts. $J+R+$ recombinants are scored as either clear (cI^-) or turbid ($cI+$); the ratio of clears to turbids is taken to be the ratio of recombination events in the *J–cI vs. cI–R* intervals. The ratio of the physical lengths of the intervals is about 2. The yield of recombinants is in all cases about 100 times lower in the $recB^-$ than the $recB+$ crosses, consistent with the loss of the major host recombination pathway. Appropriate controls indicate that the observed $ts+$ phage are recombinants that occurred in the host, as opposed to $ts+$ phage that are present in the infecting phage or arise by recombination on the plate.

red^- rec^+ λ crosses SIGNER and WEILL 1968). Thus, it is tentatively concluded that there is a *bio*-associated recombinational hot spot located in the *J–cI* interval; the hot spot activity persists when DNA synthesis is allowed, but it is dependent on a functional *recB* gene.

The effect of the *recB* gene on recombinational hot spot activity which occurs at the right end of the chromosome is examined in the companion manuscript (LAM *te al.* 1974), and has also been examined by the present authors. In a $recB^-$ host, the *A–cI/cI–R* ratio from a *b1453* cross indicates an excess of *cI–R* interval recombination events (data not shown), whereas from a red^-gam^- cross the ratio conforms to a uniform distribution of events (LAM *et al.* 1974). We don't yet understand the origin of this apparent discrepancy.

DISCUSSION

Both pure deletion and *bio*-substituted phage strains exhibit nonuniform distributions of Rec-mediated recombinants in hot spot survey crosses in which DNA synthesis is blocked. In each case the nonuniform distribution persists when DNA synthesis is allowed, and it is interpreted as the manifestation of a recombinational hot spot. The present data do not determine for either the pure deletion or the *bio* substitution case whether the hot spot is a single site, a group of sites, or an entire region of the chromosome, and the term recombinational hot spot is intended to be consistent with all of these possibilities.

Crosses employing the pure deletion phage strains exhibit strong recombinational hot spot activity in the right arm of the chromosome. The results with the *b1453* deletion indicate that the hot spot is in the *cI–R* interval. However, the nonuniform distribution in Figure 4a of the *A–cI* interval recombinants suggests that the right arm of the chromosome may be a recombinationally hot region, with the hot spot activity reaching to the left of the *cI* gene. In terms of mechanism, however, it is conceivable that all of the hot spot activity of the region is initiated at a single site and in effect spreads by virtue of the formation of heteroduplex overlap regions.

Crosses employing the *bio* substitution phage strains exhibit a recombinational hot spot located in the *J–cI* interval. The hot spot survey cross employing *bio1* further defines the locus of the hot spot to be to the right of the middle of the chromosome and to the left of the *cI* gene, close to the locus of the *bio1* insertion. However, the data are not sufficiently precise to support the tempting notion that the hot spot is located within the *bio1* insertion. On the other hand, given the very different recombinational hot spot location of the pure deletion strains as compared to the *bio* substitution strains, it seems likely that the insertion of *bio* DNA plays some central role in the associated hot spot activity. On the assumption that there is some critical site or region contained in the particular *bio* insertions employed here, it is anticipated that different (smaller) *bio* insertions might be expected to display different hot spot activity from that reported here.

It is not known from the present work whether or not the *bio*-associated (strong) *J–cI* interval hot spot activity masks (weaker) *cI–R* interval hot spot activity in the *bio* strains. Comparison of the *cI–R* interval with the *A–J* interval would be of interest.

The *bio*-associated hot spot activity is dependent on a functional *recB*. The possibility of such a dependence was rationalized with the assumption that the residual recombination in $recB^-$ strains is fundamentally different from that of $recB^+$ strains. However, the origin of the residual recombination is ill-defined, and thus it would now be of interest to test the *sbc*-activated host recombination pathways (CLARK 1973). Also, there is another potential explanation of the loss of the hot spot activity. The relevant difference may be that λ DNA replication is restricted to its early (theta) form in the $recB^+$ crosses, whereas it proceeds to its late (sigma) form in the $recB^-$ crosses (ENQUIST and SKALKA 1973). Doing $recB^+$ and $recB^-$ crosses in which DNA synthesis is blocked should help to sort out these possibilities.

It is shown in the companion manuscript (LAM *et al.* 1974) that the phage *chi* mutation causes recombinational hot spot activity in red^-gam^- strains of λ. We believe that *chi* mutations are responsible for the hot spot activity associated with the *b1319* and *b1453* deletions.

Though Rec-mediated recombinational hot spot activity has been observed, the present work does not in fact resolve the issue raised in the Introduction: Does the Rec system act uniformly along the length of the chromosome in standard (red^+ rec^+) crosses? The phage strains used here turn out to have very nonstandard features; the *chi* mutation of the pure deletion strains (LAM *et al.* 1974)

and the *bio* insertion of the substitution strains both appear to be intimately related to the recombinational hot spot activity.

Our present working hypothesis imagines that (1) the *chi* mutation results in a "*chi* sequence" of DNA which is a hot spot site for the *recB* pathway, and (2) the *coli* insertions of *bio1* and *bio69* contain "*chi* sequences." The hot spot activity observed here has a molecular basis quite different from that of the non-uniform distribution of recombination events along unreplicated chromosomes observed in the preceding paper (STAHL *et al.* 1974).

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