RECOMBINATION-DEFICIENT DELETIONS IN BACTERIOPHAGE λ AND THEIR INTERACTION WITH *CHI* MUTATIONS

DAVID HENDERSON[1] AND JON WEIL

Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

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ABSTRACT

We have isolated a new class of deletion mutants of phage lambda that extend from the prophage attachment site, *att*, into the *gam* and *cIII* genes. In this respect they are similar to certain of the λ*pbio* transducing phage, but they differ in having a low burst size and in forming minute plaques. Lytically grown stocks of the deletions contain a variable proportion of phage that produce large plaques. These have been shown to carry an additional point mutation. Similar mutations, called *chi*, have been described by LAM *et al.* (1974), who showed that they result in a hot-spot for recombination produced by the host recombination system (Rec). We show that *chi* mutations can occur at several sites in the lambda genome and produce a Rec-dependent increase in the burst size of the one deletion tested.———In addition to reducing burst size, the one deletion tested reduces synthesis of DNA and endolysin but increases production of serum blocking protein. A *chi* mutation partially restores DNA synthesis and endolysin production and reduces serum blocking protein to normal levels. Our results are consistent with the hypothesis put forward by LAM *et al.*, that *chi* enhances the frequency of Rec-promoted recombination, which provides the only pathway for production of maturable DNA in a *red*$^-$ *gam*$^-$ infection. The mechanism of the differential effect on protein production is, however, unclear.———*Chi* mutations are found to occur in DNA other than that of λ. We show that, as has been suggested elsewhere (MCMILIN, STAHL and STAHL 1974), the λ*pbio* transducing phages carry a *chi* mutation within the *E. coli* DNA substitution. A *chi* mutation also arose in a new substitution of unknown origin isolated in the course of this work.

THERE are now numerous viable deletion and substitution mutants of phage lambda which affect the central region of the genome. Their existence implies that the proteins or RNA's coded by this DNA are non-essential for lytic growth in wild-type strains of *E. coli*, either because the bacteria supply a similar function or because the phage function is truly dispensable, at least under laboratory conditions.

While the functions of many of the proteins produced by the region left of the prophage attachment site, *att*, remain to be determined, most of the coding capacity of the DNA between *att* and *cIII* is accounted for (HENDRIX 1971). As shown in Figure 1, this region contains the genes involved in prophage integra-

[1] Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125. Address reprint requests to D.H. at this address.

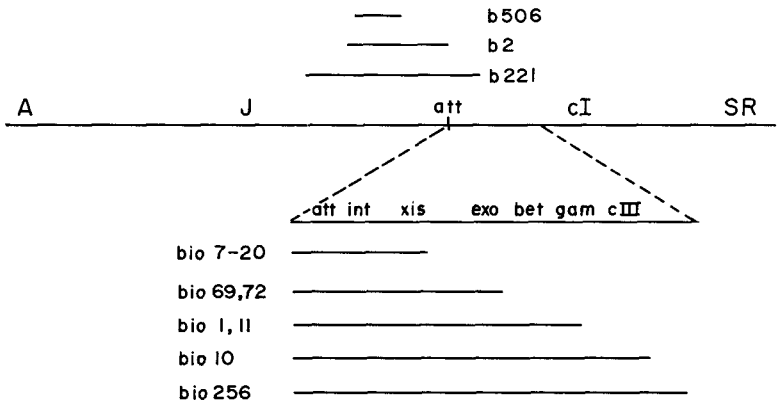


FIGURE 1.—Genetic map of phage lambda. The central region of the genome is shown expanded for clarity. The extents of the deletions and p*bio* substitutions referred to in the text are indicated above and below the map, respectively. (Adapted from DAVIDSON and SZYBALSKI 1971.)

tion and excision (*int*, ZISSLER 1967; *xis*, GUARNEROS and ECHOLS 1970) and generalized recombination (the *red* genes *exo* and *bet* (SHULMAN *et al.* 1970) and *gam* (ZISSLER, SIGNER and SCHAEFER 1971a).

All of these genes have been defined genetically by mutations and several of the proteins have now been purified and characterized. Further investigation has revealed that the growth of phage carrying mutations in some of these genes is dependent on normal host functions. For example, *red* (*exo* or *bet*) and *gam* mutants grow poorly on hosts lacking DNA polymerase I (*polA*) (N. E. MURRAY, personal communication; ZISSLER, SIGNER and SCHAEFER 1971a) and on several other classes of uncharacterized mutants termed Feb (ZISSLER, SIGNER and SCHAEFER 1971a). *Red*$^-$*gam*$^-$ double mutants exhibit the Fec$^-$ phenotype: they fail to grow on *recA* hosts (MANLY, SIGNER and RADDING 1969). Since Fec$^-$ phage make plaques on *recArecB*, *recB* and *recC* bacteria and the single *red* and *gam* mutants grow on *recA* strains, an interaction is implied between the products of the *recB* and *recC* genes and either *exo*, *bet* or *gam* protein. The *recB* and *recC* genes are known to cooperate in the production of a nuclease, RecBC nuclease (OISHI 1969; BARBOUR and CLARK 1970), whose activity is moderated by the *recA* product (WILLETTS and CLARK 1969; HOUT *et al.* 1970). UNGER and CLARK (1972) showed that there is a rapid disappearance of RecBC nuclease activity following infection by *gam*$^+$ lambda while the level remains normal in *gam*$^-$ infections. As would be expected, *gam*$^+$ infection also reduces the level of Rec-promoted recombination (UNGER, ECHOLS and CLARK 1972). It has now been shown that purified *gam* protein complexes with and inactivates RecBC nuclease *in vitro* (SAKAKI *et al.* 1973).

ENQUIST and SKALKA (1973) examined the patterns of DNA replication in infections involving various combinations of mutations in the *recA*, *recB*, *red* and *gam* genes. They concluded that the failure to form viable phage under Fec$^-$ conditions (*red*$^-$*gam*$^-$phage in a *recA* host) results from the fact that no DNA struc-

tures which can be incorporated into mature virions (i.e., concatemers or multimeric circles; STAHL *et al.* 1972) are produced. More recent experiments suggest that this failure to accumulate concatemeric DNA is at least in part due to attack by the RecBC nuclease on the nascent tail of the late replication rolling circle (M. GREENSTEIN and A. SKALKA, personal communication). At the same time, inactivation of the Red and Rec recombination systems prevents formation of multimeric DNA by recombination. For a comprehensive review of the interactions of replication and recombination in the maturation of lambda the reader is referred to STAHL *et al.* (1973) and the references cited in that paper.

The loss of *red* and *gam* function is also associated with the exclusion of lambda by the unrelated phage P2 (LINDAHL *et al.* 1970). Wild-type lambda is unable to grow on a P2 lysogen (Spi^+ phenotype), but mutants that do grow can be isolated (Spi^- mutants: LINDAHL *et al.* 1970). The available data suggest that three lambda genes are involved in the Spi^- phenotype: the *red* genes and *gam* (ZISSLER, SIGNER and SCHAEFER 1971b).

By utilizing the EDTA* resistance of deletion mutants of lambda (PARKINSON and HUSKEY 1971), we have isolated new Spi^- phage that have deletions extending from *att* into *gam* or *cIII* (Figure 1). A number of the existing λ*pbio* phage delete similar portions of the genome (see Figure 1). Unlike the *bios*, however, our new deletions make extremely small plaques. After several cycles of lytic growth, stocks of the deletions were found to contain large plaque-forming phage which carry a secondary suppressor mutation, apparently selected due to the improved growth characteristics it confers. Since we detected these suppressor mutations, apparently identical mutations have been found in stocks of red^-gam^- point mutant phage in FRANK STAHL's laboratory and have been found to act as 'hot-spots' for Rec-promoted recombination (LAM *et al.* 1974). At the suggestion of these authors, we shall refer to the suppressor mutation as *chi*.

In this paper, we characterize the new Spi^- deletions and report a variety of genetic and physiological experiments relating to the nature of the *chi* mutations and their interaction with the Fec phenotype. Our results confirm those of LAM *et al.* and extend our information regarding *chi* and its interactions.

The basic features of *chi* may be understood as follows: The low burst size and resulting small plaques of red^-gam^- phage in a wild-type host result from the fact that Rec-promoted recombination is the only route by which packageable DNA can be produced. The *chi* mutations enhance the efficiency of this route and thus improve burst size. The red^-gam^- *pbio* phages carry a *chi* hotspot within the *E. coli* DNA substitution (MCMILIN, STAHL and STAHL 1974; this paper) thus obviating the need for such a mutation in the phage DNA.

A summary of some of the data described below has been presented elsewhere (HENDERSON and WEIL 1974).

* Abbreviations used:
EDTA = ethylenediaminetetra-acetic acid
pfu = plaque-forming unit
eop = efficiency of plating
MOI = multiplicity of infection

MATERIALS AND METHODS

1) Phage and bacterial strains

The phage and bacterial strains used here are listed in Table 1. The majority of our phage stocks carry the *cI857* marker (SUSSMAN and JACOB 1962) which renders prophage thermoinducible and results in the formation of clear plaques at 37°. For convenience, this mutation is eliminated from genotype designations in the text, except where required for clarity. Hence, λ*cI857* is referred to as "wild type".

Additional strains were constructed by conventional techniques and are described in the text.

In accordance with convention, deletions isolated in this work have been given *b* prefixes.

2) Media

T broth, T agar (WEIL *et al.* 1972) and BBL Trypticase agar (STAHL and STAHL 1971) were used. KM and KG (PARKINSON 1968) were used for phage crosses and induction experiments respectively. EMB plates for lysogeny testing were prepared as described by GOTTESMAN and YARMOLINSKY (1968). SM (WEIGLE, MESELSON and PAIGEN 1959) or TM (SM without gelatin added) were used for phage dilutions.

3) Growth of phage stocks

Stocks of most phage were grown from single young plaques by confluent lysis on freshly poured T plates. Stocks of λ*red5*, λ*red329* and λ*red374* with low *red*$^+$ revertant frequencies were prepared by induction of the appropriate lysogenic bacteria in T broth. High titre stocks of λ*b1319 cI857*, free from spontaneous *chi* mutants, were made by induction of MS1138(λ*b1319 cI857*)/λ (see below for construction of this strain) and subsequent concentration of the phage by precipitation with polyethylene glycol (YAMAMOTO *et al.* 1970).

λ*imm434 cIts Sam7*, used as a source of DNA for heteroduplex preparation, was grown by induction of 594 (λ*imm434 cIts Sam7*)/λ. The cells were concentrated by centrifugation before lysis with chloroform, the phage purified on a CsCl step gradient and dialyzed into TM.

Other phage used in heteroduplex experiments were grown by confluent lysis on 10–20 T plates using C600 as host. The clarified lysates were allowed to stand overnight at 4° and the residual agar which aggregates during this period was removed by centrifugation for 15′ at 20,000 g. The phage were pelleted by centrifugation for 90′ at 25,000 rpm in a Spinco 30 rotor. Phage pellets were resuspended by soaking in 1 ml of TM 6–12 hours at 4° and residual debris from the high speed pellet was removed by centrifugation for 10′ at 10,000 g. Recovery of phage was generally about 96% and final concentrations of 4×10^{11}–10^{12} pfu/ml were obtained. These stocks were used for heteroduplex preparation without further purification except in the case of λ*b221 imm434 cIts*. This phage, due to the small size of its genome, accumulates addition and duplication mutants during growth (HENDERSON, work in progress; see also BELLETT, BUSSE and BALDWIN 1971; EMMONS, manuscript in preparation) and was purified from these variants by centrifugation in an equilibrium CsCl gradient followed by dialysis into TM.

4) Platings for plaque assay

Routine assays were made on T plates using C600 as indicator except for phage carrying *Sam* mutations which were plated on QD5003. Spi$^-$ phage without *chi* mutations were found to have slightly variable eop on T plates but had constant eop on trypticase plates, where the plaque size is larger. Except where plaque size distinction was required, these phage were plated on C600(λ*imm434*). The phage is able to recombine with the prophage, lose its deletion and consequently make a larger plaque. The eop is not affected and this procedure is purely for convenience.

5) Phage crosses

Phage crosses were carried out in KM medium. Bacteria were grown to 2×10^8/ml and mixed with an equal volume of phage in KM to give an MOI = 5 of each parent. After adsorption for 15′ and 37° the infected cells were diluted 1:100 into prewarmed KM at 37°, aerated for 70′ and chloroformed to complete lysis. Crosses with UV were performed similarly

TABLE 1

Phage and bacterial strains

1) Phage strains

Strain	Source and/or reference
λ*cI857*	(SUSSMAN and JACOB 1962)
λ*cI857 Ram5*	(SIGNER and WEIL 1968)
λ*cI857 red3 Ram5*	(SIGNER and WEIL 1968)
λ*Jam6 red3* c^+	(SIGNER and WEIL 1968)
λ*cI857 Sam6* and *Sam7*	M. HOWE (GOLDBERG and HOWE 1969)
λ*imm434cIts*	M. PTASHNE
λ*pbio1, 10* and*69*	N. E. MURRAY (MANLY, SIGNER and RADDING 1969)
λ*pbio256 cI857*	M. E. GOTTESMAN (SIGNER, MANLY and BRUNSTETTER 1969)
λ*pbio11 imm434*	E. R. SIGNER
λ*b2* c^+	(KELLENBERGER, ZICHICHI and WEIGLE 1960)
λ*b2 bio10 cI857*	I. HERSKOWITZ
λ*b506 cIam509*	J. PARKINSON (DAVIS and PARKINSON 1971)
λ*b221 c26*	J. PARKINSON (DAVIS and DAVIDSON 1968)
λ*vir*	F. STAHL
λ*reverse*	J. ZISSLER (ZISSLER, SIGNER and SCHAEFER 1971a)
Series λ*cI857 red*$^-$	E. R. SIGNER, via N. MURRAY (SHULMAN *et al.* 1970)
Series λ*pbio72* c^+ *gam*$^-$	J. ZISSLER (ZISSLER, SIGNER and SCHAEFER 1971a)

2) Bacterial strains

Strain	Relevant genotype and properties	Source or reference
C600	*supE44*: standard indicator permissive for λ*am* mutants	(APPLEYARD 1954)
C600(P2)	Plates λSpi$^-$ but not wild type	N. E. MURRAY
C600(λ*imm434*)	Indicator for λSpi$^-$chi$^+$ phage	(this lab)
CR34 Thy$^-$	*thyA6 dra1*: derived from C600, used as host for thymidine labelling	F. W. STAHL
594	*sup*°: standard indicator, non-permissive for λ*am* mutants	(CAMPBELL 1961)
594(P2)	Selective for λSpi$^-$*am*$^+$	N. E. MURRAY
QD5003	*supE57 supF58*: Permissive for *Sam* mutants	E. R. SIGNER
QR48	*supE recA1*; standard *rec*$^-$ host for genetic crosses	(SIGNER and WEIL 1968)
AB1157	Parent of AB2463, JC5743 and JC5495	(HOWARD-FLANDERS and THERIOT 1966)
AB2463	*sup37 recA13*: weak suppressor, permissive for some λ*am* mutants	(HOWARD-FLANDERS and THERIOT 1966)
JC5743	*sup37 recB21*	A. J. CLARK
JC5495	*sup37 recA13 recB21*	A. J. CLARK
ED206	*sup*° *recA56*: derived from W3110, non-permissive for λ*am*	N. WILLETTS
MS1138	cryptic lysogen in strain MSO: contains only *cos-A-J-b2* of λ	(HERSKOWITZ and SIGNER 1970)
Feb10	*supE44 polA*: derived from C600 non-permissive for *red* and *gam* mutants	(ZISSLER, SIGNER and SCHAEFER 1971a)

Mutant allele designations are those given by B. BACHMANN (1972). Most of these strains have additional auxotrophic and other mutations not relevant to this study.

except that the infected cells were irradiated with 600 ergs/mm² of UV before dilution and aeration was carried out in the dark to avoid photoreactivation.

To avoid scoring phenotypically minute plaques as recombinants in crosses where *chi*⁺ recombinants were measured, the genotype of minute plaques was verified by suspending them in SM and replating on the selective indicator. It was found that the production of phenotypically minute plaques could largely be avoided by using freshly grown plating bacteria and trypticase plates.

6) Test for lysogen formation

The EMB test of GOTTESMAN and YARMOLINSKY (1968) was used.

7) Caesium chloride density gradients

Phage in SM were mixed with an equal volume of saturated CsCl (Schwarz-Mann, Biological grade) in SM and the solution adjusted to $n_D = 1.3795$. 3.5 ml samples were centrifuged for 20–30 hrs in a Spinco SW39 or SW50.1 rotor at 25,000 rpm. Two drop fractions were collected from the bottom of the tube into 1ml aliquots of SM.

DNA content of deletion mutants was calculated using the formula of BELLETT, BUSSE and BALDWIN (1971). Wild-type lambda and λ*b2* were included in the gradients as density references. The *b2* deletion was assumed to eliminate 12.1% DNA (BELLETT, BUSSE and BALDWIN 1971).

8) Heteroduplex mapping

Heteroduplex mapping of deletions was carried out using the techniques of WESTMORELAND, SZYBALSKI and RIS (1969) as modified by DAVIS, SIMON and DAVIDSON (1971). The formamide spreading technique was used with 40% formamide in the hyperphase and 10% in the hypophase. Negatives made at 10,000× magnification with a Hitachi HUIIB microscope were traced onto paper at 10× further magnification and measured with a map measurer. In most experiments, heteroduplexes included the *imm*λ/*imm434* non-homology. The distance from the right end of the molecule to this double loop was taken to be 0.209 (WESTMORELAND, SZYBALSKI and RIS 1969) and this length used to normalize distances within each heteroduplex. We have also used φX174 RFII circular DNA, generously provided by DRS. J. S. KIM and A. FORSHEIT, as an external length standard using the ratio $\phi X/\lambda = 0.112$ (DAVIDSON and SZYBALSKI 1971). The two methods of standardization give results which do not differ significantly.

9) Construction of a lysogen of the *att*-deleted phage λ*b1319 cI857*

The rational behind the isolation of this strain is given in the RESULTS section.

MS1138(λ*b1319 cI857 chi3*) was obtained by isolating cells from the turbid centre of a plaque of λ*b1319 cI857 chi3* on the λ cryptic lysogen MS1138 at 34° (see Figure 2a). When we attempted to use this approach to construct MS1138 (λ*b1319 cI857*), however, only MS1138 (λ*b1319 cI857 chi*) derivatives were obtained. Apparently the integration of *b1319* is infrequent in MS1138 and during growth of a plaque new *chi* derivatives arise which form the majority of the stable lysogens. Results of genetic experiments suggested to us that *chi3* is near gene S (see Figure 1). We therefore crossed the *chi* mutation out of MS1138 (λ*b1319 cI857 chi3*) as follows. The lysogen was first infected at MOI = 5 with λ*cI857 Sam6*, irradiated with 600 ergs/mm² UV, and the bacteria plated on T plates at 34°. The resulting colonies were replica plated to lawns of 594 to screen for colonies that no longer released *am*⁺ phage. These colonies should be of two types: clones that are cured of the original prophage and λ*b1319 cI857 Sam6* lysogens formed by recombination (Figure 2b). The majority of clones tested were found to be non-lysogenic, but one clone was found that gave a burst of about 6 minute plaque-forming (*chi*⁺) Spi⁻ *am* phage following induction and lysis with chloroform. This lysogen was then infected with UV-irradiated λ*cI857* and clones that produced lysis when replica plated onto 594 were tested for the production of minute plaque-forming Spi⁻*am*⁺ phage. (Figure 2c). Several such clones were found and one retained for further use. The phage released on induction of this lysogen were compared with the original λ*b1319 chi3* stock by CsCl density gradient centrifugation and found to have the same density.

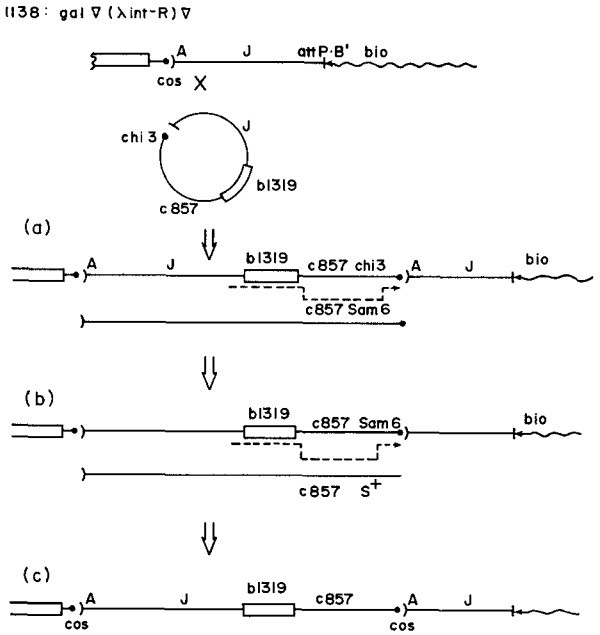


FIGURE 2.—Construction of MS1138 (*λb1319 cI857*). Construction of the lysogen is explained in the text.

10) Isolation of a phage carrying *chi3*

A phage carrying only *chi3* was isolated by crossing *λb1319 cI857 chi3* to *λcI857 Ram5*. *Am*$^+$ *b*$^+$ recombinants were selected as *am*$^+$ *Fec*$^+$ on the *su*$^-$ *recA* strain AB2463. They were tested for the presence of *chi3* in spot crosses to *λb1319 cI857* in the following manner. Individual recombinant plaques were suspended in 1 ml of SM and drops of the suspension were mixed with drops of *λb1319 cI857* at 10^6 pfu/ml on lawns of C600. After incubation overnight at 37°, phage from the spots were plated on 594(P2). Control spots made with *λb1319* alone rarely yielded large plaques, while several of the cross isolates gave 25% or more large-plaque-forming Spi$^-$ phage. One of these presumptive *chi3* isolates was tested in a quantitative liquid cross to *λb1319 cI857*. The *b1319* parent was used at an MOI = 0.01 to enhance the frequency of *b1319 chi* recombinants compared to *b1319* parental genotypes among the progeny. When the progeny were plated on 594(P2), 55% of the Spi$^-$ (*b1319*) plaques were large (i.e., *chi*-containing). Thus the original isolate is assumed to be *λchi3*.

RESULTS

Isolation of Spi$^-$ and *chi* mutations

PARKINSON and HUSKEY (1971) demonstrated that deletion mutants of lambda have increased stability relative to wild type in the presence of chelating agents such as EDTA. Using their technique, we initially isolated Spi$^-$ deletions from a heat-induced lysate of *λcI857 Ram5*. (The R mutation is used as a marker in subsequent experiments but its presence is incidental as far as isolation of the deletions is concerned.) The phage were diluted into 0.01 M EDTA, pH 8.5, and incubated at 37° for 15 minutes. Survival of plaque-forming phage was 10^{-4}. The treated phage were then plated on C600(P2). All the plaques found in these

platings were very small and occurred at a frequency corresponding to 1.5×10^{-8} of the initial titre. Phage from these plaques formed minute plaques even on non-lysogenic C600 and when grown as plate stocks (see MATERIALS AND METHODS) yielded low titre lysates (10^5–10^6 pfu/ml) containing 10–90% large-plaque-forming phage. Minute plaques from these lysates yielded heterogeneous low titre stocks when regrown, while large plaques gave homogeneous stocks with high titres (10^{10}–10^{11} pfu/ml). Both types of phage were Spi^-, Fec^-, and retained the *Ram* marker from the parent stock. Ten minute plaques were isolated from platings of the inactivated lysate and large-plaque-forming derivatives isolated from them. Some of these isolates may therefore carry the same Spi^- mutation but the large plaque characters are independent.

To test the possibility that the large plaques resulted from the selection of secondary mutations, these phage were crossed to wild-type lambda and Spi^-am^+ progeny selected on 594(P2). Nine of the ten isolates gave a mixture of large and minute plaques, indicating the segregation of a suppressing mutation. The tenth isolate, *b1262*, gave very few minute plaques and represents a special case, as will be shown below. These crosses will be discussed in greater detail in the section dealing with the genetic properties of the suppressors and are mentioned here only to point out that most of the phage used to characterize the Spi^- mutations carry these additional mutations, which we shall call *chi* (see beginning of paper).

A second set of deletion mutants was isolated starting with phage strain *λcI857 chi3*, whose *chi* mtuation is derived from one of the isolates described above (see MATERIALS AND METHODS). We shall now describe in detail the procedures used to isolate these phage because they are of general technical interest.

During the course of these experiments, we have developed a method for the direct selection of deletion mutants which avoids the recycling of EDTA-inactivated phage used by PARKINSON and HUSKEY (1971). The phage are inactivated once in EDTA in liquid to reduce the non-deletion background and are then plated on trypticase plates containing 10^{-3} M EDTA, pH 7. Neither wild-type lambda nor *λb1451*, a strain with a 1.8% deletion isolated in the present study, will grow on these plates. However, *λimm434*, with a deletion of 2.2% (WESTMORELAND, SZYBALSKI and RIS 1969), and all phage with larger deletions have normal efficiency of plating. This selection method has the potential of allowing isolation of deletions with reduced burst size that would be selected against with the recycling technique of PARKINSON and HUSKEY (1971). It also allows the direct measurement of deletion frequencies, since the phenotypically EDTA-resistant particles described by PARKINSON and HUSKEY do not form plaques on EDTA plates.

PARKINSON and HUSKEY found that all the deletion mutants isolated from UV-induced lysates of lambda originated at the attachment site. They observed that vegetatively grown stocks show a lower frequency of deletions and in addition containing some deletions that do not originate at *att*. Using EDTA plates for selection, we have found that heat-induced lysates also contain only deletions originating at *att*, but at a frequency comparable to that of vegetatively grown stocks (about 5×10^{-6}). This suggests that the higher frequency of deletions

found after UV induction (10^{-4}) is a function of the UV irradiation, while the types of deletion formed are a consequence of the events associated with prophage excision. We reasoned that irradiation of vegetatively growing phage should result in an enhancement of deletion frequencies, including those that do not originate at *att*. The following experiment was done to test this idea, with the aim of isolating *att*$^+$ Spi$^-$ deletions. C600 was grown to 2×10^8/ml in KM and infected at an MOI=10 with λ*cI857 chi3*. After adsorption for 15′ at 37°, the infected cells were irradiated with 600 ergs/mm^2 UV and the culture divided into several tubes. These were aerated in the dark for 70′ at 37° after which time lysis was completed with chloroform and debris removed by centrifugation.

The irradiation was found to increase the total deletion frequency about 40× from 6×10^{-6} for the lytically grown λ*cI857 chi3* used for infection, to 2.3×10^{-4}. The Spi$^-$ frequency was increased about 30×, to 5.2×10^{-6}. As would be expected, many of the Spi$^-$ mutants derived from λ*cI857 chi3* make large plaques. Minute plaques are, however, still found and it is possible that these represent mutants not detectable in wild-type lysates due to their poor plaque-forming ability in the absence of *chi*. This class of minute plaque-forming phage has not been investigated.

The deletion-enriched λ*cI857 chi3* lysates were inactivated with EDTA, plated on 594(P2) at 34° and large turbid (*cIII*$^+$) plaques tested for their ability to make stable lysogens (see MATERIALS AND METHODS). Four independent mutants were kept—two that made stable lysogens and were presumably *att*$^+$ (*b1451 chi3*, *b1452 chi3*) and two that did not lysogenize, presumably *att*-deleted (*b1453 chi3*, *b1454 chi3*).

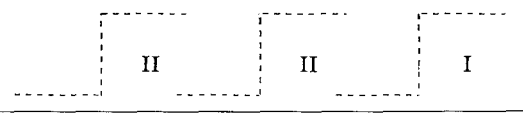
General characteristics of Spi$^-$ mutants

Initial characterization of the presumed deletions was performed on the isolates from λ*cI857 Ram5*. All ten isolates were found to be unable to lysogenize both C600 and the cryptic lysogen U427, indicating loss of at least the right-hand recognition region of *att* (*RER*, PARKINSON 1971 and see below). As expected, the phage were also Fec$^-$, i.e., failed to plate on the *su*$^+$ *recA* strain QR48 (MANLY, SIGNER and RADDING 1969; see also beginning of this paper).

In order to test the prediction that the phage are deleted for the attachment site and the recombination genes, quantitative genetic crosses were performed with four of the mutants in a *recA* host (SIGNER and WEIL 1968). Results of these crosses, together with the appropriate controls, are shown in Table 2. As anticipated, all four phage proved to be deficient in Red-promoted recombination. They were, however, found to differ in their behavior in Int-promoted recombination. Three of the isolates (λ*b1165*, λ*b1169* and λ*b1319*) gave no detectable Int-promoted recombinants, while λ*b1262* gave a low, but significant, number. This difference can most easily be explained as resulting from differences in the extent of the deletions in the attachment region. The structure of the attachment site of lambda has been analyzed by PARKINSON (1971), who proposed that there are two recognition regions flanking the crossover point where the site-specific recombination occurs: *REL* on the left-hand side and *RER* on the right. Phage

TABLE 2

Results of crosses between Spi⁻ chi Ram5 isolates and λJam6 red3 c⁺ in QR48.



Second parent	% Recombinants observed* J to c	c to R
λ*cI857 Ram5* (control)	0.16	0.41
λ*red3 cI857 Ram5* (control)	0.8	<0.003
λ*b1165 cI857 chi1 Ram5*	<0.001	<0.005
λ*b1169 cI857 chi2 Ram5*	<0.001	<0.004
λ*b1262 cI857 chi4 Ram5*	0.02	<0.005
λ*b1319 cI857 chi3 Ram5*	<0.001	<0.004

Am⁺ recombinants were scored for clear (*c*⁻: recombination between *c* and *R*, marked *I* in diagram) or turbid (*c*⁺: recombination between *J* and *c*, marked II in diagram). In a *recA* host, recombination in the *c–R* interval (I) in produced only by Red, while in the *J–c* interval both Red and Int produce recombinants (See for example SIGNER and WEIL 1968; WEIL and SIGNER 1968). Since the *Jam6* parent is *red*⁻, *c–R* recombination indicates a functional *red* gene in the *Ram5* parent (line 1). In the absence of Red function, recombination between *J* and *c* indicates a functional *att* in the *Ram5* parent (line 2).

* 200×pfu on 594/pfu on C600, at 37°, averaged from duplicate crosses.

deleted for one or both of these elements, or carrying a substitution of the complemenatry element from the bacterial attachment site, give different and characteristic recombination frequencies in Int-promoted crosses (PARKINSON 1971). Comparison of our data with PARKINSON's suggests that λ*b1262* has retained *REL* but is deleted or substituted for *RER* while the remaining phage have lost both *REL* and *RER*.

At 34°, the permissive temperature for *cI857*, the plaques of all the isolates were incompletely turbid, suggesting that the deletions might extend as far as *cIII*. To test this possibility, the four strains used in the quantitative crosses described above were streaked across a streak of λ*cIII67* on a lawn of C600 and incubated at 37°. *cIII*⁺ phage complement the *cIII67* mutant and produce a turbid area of lysogeny where the streaks intersect. The Spi⁻ phage failed to complement and are assumed to have a *cIII* defect. Appropriate crosses have also failed to detect segregation of Spi and *cIII* in λ*b1319* (data not shown).

The above results are consistent with the hypothesis that the Spi⁻ defects are deletions or substitutions extending from the attachment site to *cIII* or beyond. In order to verify this we have performed heteroduplex mapping experiments with these four phage (see below).

Characterization of Spi⁻ mutants isolated from λ*chi3*

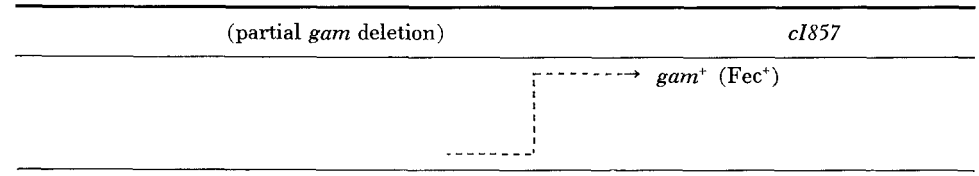
Caesium chloride density gradients verified that λ*b1451*, λ*b1453* and λ*b1454* had all lost DNA (see data shown in Table 5). λ*b1452* was found to have the

same density as wild-type lambda and recombined with all the *red* and *gam* point mutants tested. The nature of the Spi⁻ mutation in this phage is not known and it has not been further investigated.

Because they were isolated as turbid plaque-formers, it was assumed that these deletions would end to the left of *cIII*, possibly in the *gam* gene. Therefore, the right-hand ends of the deletions were mapped with respect to mutants in *gam* by crossing the deletion strains to λ*bios* carrying different *gam* mutations. Since the λ*bio gam*⁻ strains are Fec⁻ while their *gam*⁺ derivatives are not, the generation of Fec⁺ recombinants in such a cross indicates that the deletion does not extend as far as the *gam* mutation against which it was crossed (see the figure in Table 3). The results indicate that *b1453* and *b1454* have distinct endpoints within the *gam* gene (Table 3). *b1451* was found to recombine with all the *gam* mutants tested and therefore either does not delete any of the *gam* gene or does not extend into *gam* as far as the first mutant tested.

We shall show below that the right end of *b1451* lies within the *exo* gene. To account for its Spi⁻ phenotype it was thus presumed that it must also carry a point mutation in *gam*. This leads to the prediction that in a cross to a *gam*⁺ phage, a *Fec*⁺ *red*⁻ recombinant carrying only the deletion should be generated (see Figure 3a). To test this, an *Ram5* derivative of the Spi⁻ phage was crossed to λ*b2 c*⁺. The progeny of the cross were run on a CsCl gradient and assayed on C600 for total phage and on the *su*⁻ *recA* strain ED206 for Fec⁺ *am*⁺ recombinants. As shown in Figure 3a, two classes of Fec⁺ *am*⁺ *c*⁺ phage should emerge from the cross in addition to the *b2* parent: recombinants produced by an exchange between the two deletions, which will be of wild-type density and *red*⁺,

TABLE 3



Deletion	*bio*		*gam*⁻		*c*⁺	
	gam mutation					
	am207	5	ts23	ts302	am301	am210
b1451	0.036	0.016	0.005	0.006	0.030	0.025
(c⁺/c⁻)*	(2.02)	(1.84)	(1.73)	(0.56)	(0.52)	(0.49)
b1453	(NT)†	(NT)	$<10^{-5}$	$<10^{-5}$	0.02	0.01
b1454	(NT)	(NT)	$<10^{-5}$	$<10^{-5}$	$<4 \times 10^{-5}$	0.01

Crosses were performed between the deletions indicated and the *gam* mutants in *bio69* or *bio72*. The mutants *am207*, *5*, *ts23* and *am210* have been described (ZISSLER, SIGNER and SCHAEFER 1971a). The mutants *ts302* and *am301* were isolated by us as conditional Spi⁻ mutants of *bio69* (see also ZISSLER, SIGNER and SCHAEFER 1971a). Fec⁺ recombinants were detected on ED206 at 37°, or 42° for the *ts* mutants. Figures are recombinant frequencies for the exchange between the deletion and *gam* (see Figure above) = 200× Fec⁺ pfu on ED206/total pfu on C600.

* Figures in parentheses indicate the ratio of c^+ to c^- Fec⁺ recombinants (see text).

† (NT) = not tested.

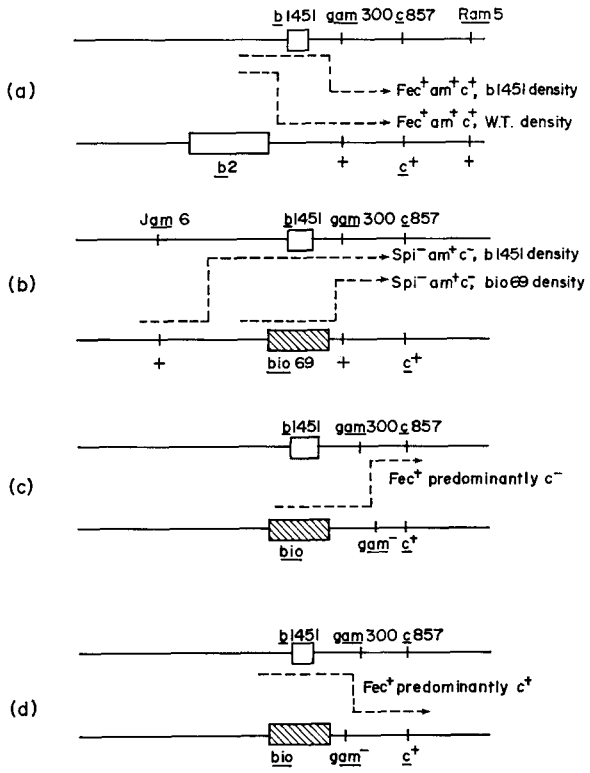


FIGURE 3.—Behavior of λ*b1451 gam300 chi3* in genetic crosses.

and phage recombined between the *b1451* deletion and the proposed *gam* mutation, which will have the density of *b*1451 and be *red*⁻. Two classes of turbid plaques were found on ED206 in addition to the λ*b2* c^+ parent: large plaques with an average density greater than *b1451* and smaller ones that had an average density very close to the *b1451* parent. Several of the latter were repurified and found to be *red*⁻ and Spi⁺. One of these was retained as λ*b1451* c^+ and used in the subsequent mapping experiments. The isolation of a *red*⁻ Fec⁺ phage with the same density as the original *b1451 chi*3 isolate supports the hypothesis that this phage is actually a double mutant.

To verify the presence of the *gam* point mutation, a *Jam6* derivative of the Spi⁻ phage was crossed to λ*bio69* c^+ (Figure 3b). It is predicted that this should generate two types of Spi⁻ *am*⁺ c^- recombinants: one of *b1451* density and one of *bio69* density. Since *b1451* and *bio69* have quite similar DNA contents, the two parental phage formed overlapping peaks when the cross lysate was run in a CsCl gradient, but they could be distinguished by the *cI857* marker in the *b1451* phage. A single peak of Spi⁻ *am*⁺ recombinants was found at the density of *b1451*. This is not unexpected. Since the distance between *J* and *bio* is much larger than that between *bio* and *gam*, the less frequent *bio69 gam* recombinants might be undetectable under the larger *b1451 gam am*⁺ peak. The phage from

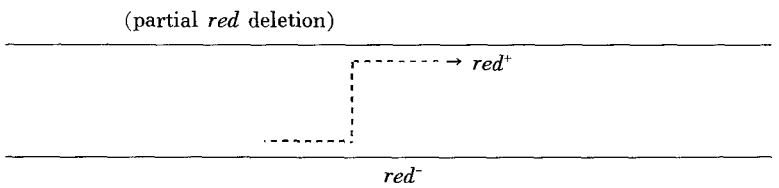
the leading edge of the (denser) *bio69* peak were therefore run in a second CsCl gradient. In this gradient, a peak of Spi⁻ *am*⁺ *c*⁻ phage of *bio69* density was found. This result is consistent with there being a mutation in *gam* separable from the deletion in the original *b1451* isolate, which we shall now refer to as *λb1451 gam300 chi3*.

Analysis of the crosses of this phage to the *λbio gam*⁻ phage indicates that the *gam300* mutation probably lies between *gamts23* and *gamts302* (Table 3). The order of the markers shown in Table 3 is derived as follows: ZISSLER, SIGNER and SCHAEFER (1971a) demonstrated the order *gamam207–gam5–(gamts23, gamam-210)*. The crosses using *b1453* and *b1454* indicate that *gamts23* is left of *gamam210* and that *gamam301* lies between *gamts302* and *gamam210*, but leave *gamts302* unordered with respect to *gamts23* and *gam5*. The remainder of the order can be deduced from the crosses to *λb1451 gam300 chi3* itself. Since the crosses were performed between *λbio gam⁻c⁺* and *λb1451 gam300 cI857 chi3*, the c⁺/c⁻ ratio of the Fec⁺ recombinants can be used to determine the order of the two *gam* mutations, assuming that the majority of Fec⁺ recombinants arose by single exchanges. If *gam300* is left of the tester *gam*⁻, the majority of Fec⁺ recombinants should be *c*⁻ (Figure 3c), while if *gam300* is right of the tester, the recombinants will be predominantly *c*⁺ (Figure 3d). As shown in Table 3, the break in the c⁺/c⁻ ratio occurs between *gamts23* and *gamts302*. It should also be noticed that the recombination frequency for formation of Fec⁺ recombinants is at a minimum for these two markers, suggesting that they are closest to *gam300*. Thus the results are self-consistent and suggest the order *gamts23–gam300–gamts302*.

The endpoints of the *b1451* deletion were mapped genetically by crossing to various *red* point mutants and selecting for *red*⁺ recombinants on a *polA* strain.

TABLE 4

Mapping of b1451 *against* red *point mutants*



	exo					bet		
red mutant #	(5	329)	1	314	324	525	3	374
	$<3 \times 10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<3 \times 10^{-5}$	1.8×10^{-4}	0.004	0.017	0.082

Red⁺ recombinants were selected on the *polA* strain Feb10 (ZISSLER, SIGNER and SCHAEFER 1971a) at 37°, with two exceptions. The cross to the *ts* mutant *red374* was plated at 42° and the cross to *red5*, which is slightly leaky at 37°, was plated at 39°. Figures are recombinant frequencies between the deletion and point mutation (*red⁺c⁺* recombinants). Scoring of this recombinant rather than total *red*⁺ phage was used to avoid the *red⁺c⁻* revertants of the point mutant parent which are present at levels close to that of the recombinants detected.

The order of the point mutants is taken from SHULMAN *et al.* (1970). Results are the averages from duplicate crosses except to mutants in *bet*, which were performed once only.

Results of these crosses are shown in Table 4. The right end of *b1451* is toward the right end of *exo*, between *red314* and *red324*, while the left end is left of the unordered mutants *red5* and *red329*.

Heteroduplex mapping

The physical positions of five of the Spi⁻ mutants and the *red*⁻ deletion *b1451* were measured in heteroduplex studies. Measurements were made of heteroduplexes of the Spi⁻ phage to both λ*imm434* and λ*b506* DNA and of λ*b1451* to λ*b221 imm434*. The phage were found to carry deletions, with the exception of *b1262*, which has a substitution of unknown origin.

Heteroduplexes to λ*imm434* were used to measure the positions of the right ends of Spi⁻ deletions and the *b1262* substitution. The distance from the right end of the *imm434/imm*λ substitution loop to the right end of the molecule was used as a length standard. The left ends of the deletions were measured in heteroduplexes to λ*b506* using φXRFII circles as standards (see MATERIALS AND METHODS). Using the position of *b506* reported by DAVIS and PARKINSON (1971), the left ends of the deletions appeared to map too far to the right of the accepted position of *att*, which would be inconsistent with the genetic properties of the phage. The right end of *b506* was therefore remapped using heteroduplexes to λ*bio11 imm434*. Lengths were standardized using both the *imm*–right end distance and φX RF DNA. The position of *b506* was then calculated assuming that the *bio11* substitution originates at *att* and that this point is at 0.573 (HRADECNA and SZYBALSKI 1969). This gives the right end of *b506* as 0.500 ± 0.003 with both methods of standardization, rather than the value of 0.522 previously published. This new value was used to determine the deletion and substitution endpoints shown in Table 5 and Figure 4.

The left endpoints of the deletions found by heteroduplex mapping are consistent with the behavior of these phage in Int-promoted recombination. *b1165* clearly ends left of the crossover point ($P < 0.001$), but our data for

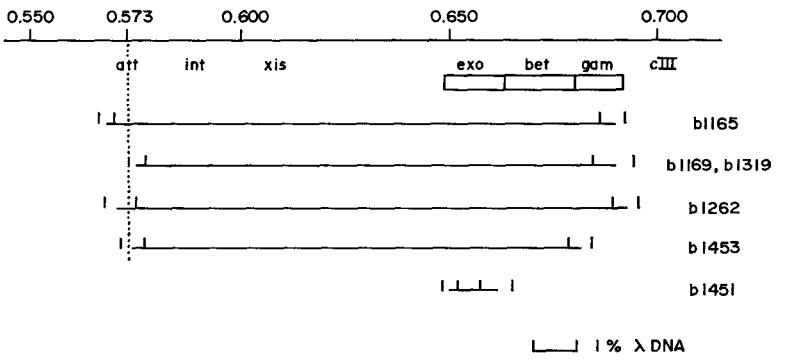


FIGURE 4.—Deletion map of the central region of the lambda genome. The deletions mapped by electron microscopy are shown on a scale of 0–1.0 for the lambda genome. Vertical bars indicate the standard deviations in the electron microscopic measurements. The size and position of the *exo*, *bet* and *gam* genes are shown (see DISCUSSION).

TABLE 5

Summary of physical properties of deletions

Deletion	Size from† CsCl gradient	EM heteroduplex results* Left end	Right end	Size from† EM results
b1165	(ND)§	0.568 ± 0.002 (13)	0.692 ± 0.003 (15)	14.0 ± 0.5
b1169	(ND)	0.575 ± 0.002 (13)	0.686 ± 0.006 (16)	11.1 ± 0.8
b1262	4.7	0.571 ± 0.004 (18)	0.692 ± 0.003 (12)	12.1 ± 0.7‡
b1319	11.7	0.572 ± 0.030 (16)	0.689 ± 0.005 (16)	11.7 ± 0.5
b1451	1.8	0.650 ± 0.002 (17)	0.661 ± 0.004 (17)	1.1 ± 0.6
b1453	11.0	0.574 ± 0.003 (12)	0.681 ± 0.003 (24)	10.7 ± 0.6
b1454	11.2	(ND)	(ND)	(ND)

* Figures represent means and standard deviations of the positions of the ends of the deletions calculated as described in the text. Figures in parentheses are the numbers of molecules measured. Left endpoints of the deletions were measured against *b506* (for *b1165*, *b1169*, *b1262* and *b1453*) or *b221* (for *b1451*) as described in the text. *b1169* and *b1319* are very similar in size. Since they are not independent isolates they may be the same deletion and *b1319* therefore was not heteroduplexed to *b506*. The left endpoint given for *b1319* above was measured against the left end of the molecule in heteroduplexes to λ*imm434*.

† % of lambda DNA deleted

‡ Measurements represent the endpoints of the substitution and the deletion of lambda DNA in this phage. The length of substituted DNA is 7.4 ± 0.7% of lambda.

§ (ND) = not determined

the remaining phages do not critically distinguish left endpoints at the crossover point or within *REL*. Based on the genetic experiments, it is assumed that *b1262* leaves *REL* intact while *b1165*, *b1169* and *b1319* delete at least enough of *REL* to render it inoperative in Int-promoted recombination.

We have been unable to identify the origin of the substitution carried by λ*b1262*. The DNA is not homologous to that carried by λ*bio10*. We have also heteroduplexed λ*b1262* to λ*reverse*, which carries a much larger substitution in the central region of the genome (GOTTESMAN *et al.* 1974). The *b1262* substitution is not homologous to any part of this DNA. It is possible that the phage was created by excision of a prophage inserted at an abnormal site in the bacterial genome. Such insertion is, however, very rare when the host has a normal attachment site, as was the case in the strain used to grow the parental stock for these experiments (SHIMADA, WEISBERG and GOTTESMAN 1972). It seems unlikely that the lysogen used was abnormal, but we have not checked to see if normal numbers of λ*gal* or λ*bio* transducing particles are generated.

b1451 was mapped only in heteroduplexes to λ*b221 imm434*, using the published position of 0.629 for the right end of *b221* (DAVIS and DAVIDSON 1968). The same mean position and standard deviations were found for the left and right ends of *b1451* using both internal and φX RF length standards. The position of the deletion is consistent with the genetic mapping data.

Genetic location of *chi* mutations

Representative results from the previously discussed crosses between the Spi⁻ *chi Ram5* isolates and wild-type phage are shown in Table 6. The crosses

TABLE 6

Frequency of recombinant production in crosses of Spi⁻chi Ram5 phage to wild type

am⁺ Recombinant scored	Phage isolate*						
	1262	1169	1102	1165	1342	1111	1319
Minute (chi⁺)	<0.03	1.5	3.8	3.8	4.8	4.0	6.1
Large (chi)	7.1	7.8	1.6	1.2	1.4	1.0	0.9
Total	7.1	9.3	5.4	5.0	6.2	5.0	7.0

The figures shown are recombinant frequencies = 200 × pfu on 594(P2)/pfu on C600 and are averaged from at least two crosses.

* Spi⁻ *chi Ram5* isolates were given isolation numbers as shown. These numbers were used to designate deletions in phage subsequently shown to carry them. *Chi* mutations were numbered separately, as shown in the text.

were plated on 594(P2) to select for recombinants between the Spi⁻ mutation and *Ram5* and the *chi* marker was scored by plaque size. There are two aspects to be noted in these results.

Firstly, the ratio of *chi*⁺ to *chi*⁻ recombinants is highly variable. This is formally consistent with the *chi* mutations having different locations, with a high *chi*⁺/*chi*⁻ ratio implying that the given *chi* mutation lies relatively far to the right on the genetic map. However, it should be borne in mind that *chi* mutations apparently affect recombination and the differences may therefore reflect 'marker effects' rather than map location. Secondly, the frequency of Spi⁻*am*⁺ progeny varies slightly in the different crosses but is higher than that seen in crosses where *chi* is not present (see, for example, SIGNER and WEIL 1968). This is consistent with the observation of LAM *et al.* (1974) that *chi* introduces a hot-spot for recombination but it is not conclusive because the relevant control was not run in our experiments.

In order to determine if the *chi* mutations are actually at different sites, crosses were performed to look for recombination between different *chi*s. The *am*⁺ derivatives of *b1165chi1*, *b1169chi2* and *b1319chi3* were isolated from the crosses of the original Spi⁻*chi Ram5* phage to wild-type and *Jam6* derivatives prepared from them. It was assumed that the large plaque Spi⁻ *Jam6* recombinants from these crosses would retain the original *chi* mutation. In order to verify this, the *Jam6* phage were crossed to their *Ram5* progenitors. No *am*⁺*chi*⁺ recombinants were found.

The *Jam6* and *Ram5* derivatives of phage carrying different *chi* mutations were crossed and *am*⁺ progeny scored for *chi* by plaque size. The crosses were performed with the selective markers in both orientations with respect to the *chi* mutations (Figure 5a and b). In Figure 5a, single exchanges in intervals 1 and 3 generate *chi am*⁺ (large-plaque-forming) recombinants and single exchanges in interval 2 produce *chi*⁺*am*⁺ (minute-plaque-forming) recombinants. In Figure 5b, single exchanges in all three intervals produce *chi am*⁺ recombinants. Hence, assuming that the *chi* mutations do not produce an excess of multiple over single exchanges, the relative order of pairs of *chi* mutations can be deduced

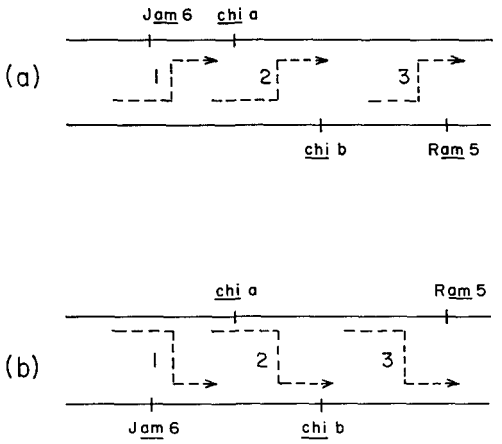


FIGURE 5.—Theoretical behavior of two *chi* mutations in crosses of *Jam6* × *Ram5* derivatives. For convenience, the *chi* mutations are shown between *J* and *R*. The numbers indicate the intervals in which recombination can be scored. The types of recombinant produced by each possible event are described in the text.

from such pairs of crosses. The results of crosses with *chi* mutations *1*, *2*, and *3* are shown in Table 7, column (a).

The three *chi* mutations recombine with each other and are found to segregate qualitatively as expected from the map order suggested by the crosses to wild type. That is, they appear to be ordered *chi2–chi1–chi3*. In the crosses *chi1* × *chi2* and *chi2* × *chi3* (Crosses 1–4), the majority of exchanges between *J* and *R* occur between the two chis. In the crosses of *chi1* × *chi3* (crosses 5 and 6) however, the majority of recombinants are produced by exchanges outside the *chi1–chi3* interval. In addition, the relatively high frequency of $am^{+}chi^{+}$ recombinants in cross 6 compared to cross 5 indicates that there is negative interference associated with the exchanges between *chi1* and *chi3*. These results suggest that *chi1* and *chi3* are closer to each other than they are to *chi2*. Since *rec*-promoted exchanges

TABLE 7

Frequency of recombinant production in crosses between different chi *mutants*

Cross	λ*chi⁻Ram5**	λ*chi⁻Jam6**	*J-R* recombinant frequency† (a) In rec⁺ host *chi*	(a) In rec⁺ host *chi⁺*	(b) In *recB host*‡ *chi*	(b) In *recB host*‡ *chi⁺*
1	*chi1*	*chi2*	0.84	10.4	0.04	0.30
2	*chi2*	*chi1*	5.4	0.18	0.21	0.009
3	*chi3*	*chi2*	0.62	7.8	0.053	0.59
4	*chi2*	*chi3*	3.0	0.15	0.24	0.014
5	*chi3*	*chi1*	7.3	0.81	0.39	0.015
6	*chi1*	*chi3*	5.2	0.30	0.22	0.006

* Both phage carry a Spi⁻ deletion, as described in the text.
† 200 × pfu on 594/pfu on C600.
‡ the *recB* host used was JC5743.
Recombinant frequencies shown are averaged from duplicate crosses.

occur as clusters (NORKIN 1970; see also SIGNER 1971), *chi1* and *chi3* are presumably separated by a distance less than the length of the average exchange cluster.

Because of the uncertainty introduced by the nature of *chi*, we are unable to order the *chi* mutations with respect to *J* and *R*. It should be noted, however, that they behave as if *chi2* is relatively close to *J* and *chi3* close to *R*. The inference that *chi1* and *chi3* are closer to each other than to *chi2* can be assumed to be correct, unless the *chi* mutations differ in their ability to influence multiple exchanges.

The following aspects of the data also deserve attention. Firstly, the recombinant frequencies in the *J–R* interval vary for the different combinations of *chi* mutants tested. This is consistent with the variations in Spi-*R* recombinant frequencies shown in Table 6, but it should be pointed out that in those crosses Red was active as well as Rec and the activity of the host Rec system would be reduced by the phage *gam* protein from the wild-type parent (UNGER. ECHOLS and CLARK 1972). The variation in *J–R* recombination may indicate either that *chi* mutations vary in their ability to stimulate Rec or that the presence of two different *chi* mutations on the recombining genomes in some cases has a further influence on recombination. We should like to stress that, while small, the variations in recombinant frequencies are reproducible.

Secondly, the ratio of recombinant frequencies for the pairs of crosses involving the same *chi* mutations, but with reversed orientation of the selective markers, is in all cases approximately 0.5. A higher recombinant frequency is observed in crosses where the *Ram5* parent carries the right-most of the two *chi* mutations in question (Figure 5a; Table 7, crosses 1, 3, and 5). The simplest interpretation of this observation is that there is an asymmetry in recombination events associated with *chi* mutations. An asymmetry was also observed by LAM *et al.* (1974) in crosses where only one parent carried a *chi* mutation.

chi mutations are acted on by the RecF pathway

It has been shown that there are several pathways of genetic recombination in *E. coli*. The major pathway in wild-type cells is that eliminated by mutations in the *recB* or *recC* genes: the RecBC pathway. A second pathway, RecF (HORII and CLARK 1973), is active at a low level in *recB* and *recC* mutants. In order to determine whether *chi* mutations are active with the RecF pathway, the *chi* × *chi* crosses were repeated in a *recB* host. Results of these crosses are shown in Table 7, column (b).

While the recombinant frequency is substantially reduced, there are variations in recombinant frequency between *J* and *R* and asymmetry in reciprocal crosses which closely parallel those in the *rec*$^+$ host. Since the variations and asymmetries are most easily explained as being due to interactions between the *chi* mutations and the recombination systems, it would appear that the RecF pathway interacts with *chi* mutations in a manner similar to the RecBC pathway.

chi mutations in Spi⁻ substitution mutants

We found it striking that the previously isolated Spi⁻ λp*bios* had not been reported to carry *chi* mutations. A possible explanation for this was suggested by McMILIN, STAHL and STAHL (1974). They found that *bio1* and *bio69* carry a recombination hot-spot near the center of the genome, in contrast to the deletion mutants *b1319* and *b1453* which have hot-spots near the right end. This could be interpreted to mean that there is a hot-spot (*chi*) within or near the *bio* DNA substitution.

A number of crosses were performed to try and detect segregation of *chi* from λ*bio1*, λ*bio10* and λ*bio256*. Our first approach was to cross the *Ram5* derivatives of the *bios* to wild type at an MOI=5 of each parent. Spi⁻*am*⁺ progeny were then screened for minute plaques; none were found (Table 8, lines 1 and 2). Further crosses were then performed using an MOI=5 of wild type and 0.01 of *am*⁺*bio*. As was shown by WOLLMAN and JACOB (1954), the ratio of recombinant to minority parental genotypes among the progeny of such a cross is high, and we therefore increased the sensitivity with which recombinants could be detected when plating for Spi⁻ progeny. To further increase recombination, the infected cells were irradiated with UV (see MATERIALS AND METHODS). In the cross λ*cI857* × λ*bio1* c^+, this procedure gave 54% recombinant frequency in the *bio–cI* region. Despite this enhanced recombinant frequency, the majority of crosses failed to yield minute plaque-forming recombinants (Table 8, lines 3–4). In two crosses, however, one of λ*bio10* and one of λ*bio256*, single minute plaques were found.

TABLE 8

Behavior of chi *in Spi⁻ substitution mutants and their derivatives*

	Cross* conditions	Frequency of minute Spi⁻ plaques among total progeny phage	Recombinant frequency Spi–*R* (%)†
bio1 c^+ *Ram5*	(b)	$<1.7 \times 10^{-4}$	5.0
bio10 c^- *Ram5*	(b)	$<1.3 \times 10^{-4}$	7.4
bio1 c^+	(a)	$<2.5 \times 10^{-5}$	
bio10 c^-	(a)	$<3.4 \times 10^{-5}$	
bio256 cI857 (1)	(a)	$<2.8 \times 10^{-5}$	
(2)	(a)	$<1.2 \times 10^{-5}$	
b10–1 chi	(a)	5.2×10^{-3}	
b256–1 chi	(b)	7.6×10^{-4}	
b1262 chi4 Ram5	(c)	6.7×10^{-4}	20.5
b1262–1 chi10 Ram5	(d)	1.6×10^{-2}	8.5
b1262–2 chi18 Ram5	(d)	1.5×10^{-2}	7.9

* All crosses were against wild type and performed in C600.
(a) low MOI + UV, average of two crosses
(b) high MOI, one cross only
(c) high MOI + UV, average of three crosses
(d) high MOI, average of two crosses
In crosses where the Spi⁻ parent carries *Ram5*, the frequency of minute Spi⁻*am*⁺ plaques is shown.

† 200× pfu on 594(P2)/total pfu on C600.

These two phage were purified, allowed to pick up a new *chi* mutation, and their properties investigated. It was found that neither transduced *bio* (data not shown) and that they segregated *chi* when crossed to wild type (Table 8, *b10-1 chi* and *b256-1 chi*, lines 6 and 7). When heteroduplexed to λ*bio11 imm434* and λ*b2bio10*, it was found that they carried simple deletions with no remnant of the original *bio* substitutions. It is thus probable that these two phage represent spontaneous Spi⁻ deletions that were present in the wild-type phage stock used in the crosses.

The Spi⁻ substitution mutant λ*b1262 chi4 Ram5* was found to behave differently from the p*bio*s. Although the frequency is low, UV crosses to wildtype at MOI=5 of each parent were found to give minute plaque Spi⁻ *am*⁺ recombinants (Table 8, line 8). Two minute plaques from each of two crosses were picked and new *chi* derivatives isolated. The *Ram5* derivatives of two of these isolates, which were from the same cross and therefore possibly identical, were made, crossed to wild type and found to segregate *chi* (Table 8, lines 9 and 10, *b1262-1 chi10 Ram5* and *b1262-2 chi18 Ram5*). All four isolates were heteroduplexed to λ*imm434* and found to carry pure deletions. We do not know if the formation of these deletions occurred spontaneously during growth of *b1262* or if the presence of wild-type genomes in the cross was also involved.

We interpret all of these results to mean that in the *bio* transducing phage and *b1262* the *chi* mutation is situated within the substituted DNA. It is not clear at present whether the *chi* within *bio* pre-exists in the *E. coli* genome or if the p*bio* phage pick this up subsequent to their formation. The following facts, however, support the hypothesis that the mutation pre-exists in the *E. coli* genome. Firstly, all the Spi⁻ λ*bio*s tested contain their *chi* mutations within the *bio* DNA (McMilin, Stahl and Stahl 1974; this paper) rather than at one of the several sites available in the lambda genome. Secondly, even those λ*bio*s that are Spi⁺ and thus do not require a *chi* to grow well appear to carry a *chi* in the *bio* DNA. There are two lines of evidence to support this contention. Phage such as λ*bio69* are rendered Spi⁻ by a mutation in *gam* (Zissler, Signer and Schaefer 1971 b). We have observed that spontaneous Spi⁻ mutants in stocks of λ*bio69* always make large plaques, suggesting that *chi* does not need to be acquired by a second mutational event (unpublished experiments). It has also been shown that derivatives of λ*bio69* have a recombination hot-spot associated with the *bio* DNA (McMilin, Stahl and Stahl, 1974).

In the case of *b1262*, we know that the phage made minute plaques when first isolated. We have not determined if *chi* mutations always arise within the *b1262* substitution or if they can occur elsewhere in the genome as well.

Effects of Spi⁻ deletions and *chi* on phage development

The *b1319* deletion and its associated *chi3* mutation were used to investigate the effects of a Spi⁻ deletion and *chi* on phage development.

Since plate stocks grown from minute *b1319* plaques always have very low titres and a high frequency of *chi* mutations, it was first necessary to devise a method of growing relatively homogeneous stocks of the unsuppressed deletion.

In principle, this could be accomplished by making a lysogen of the phage and inducing it, since passive replication in the prophage state would prevent selection for *chi* mutants. To circumvent the fact that *b1319* is *int*$^-$*xis*$^-$ and has a partially deleted attachment site, we lysogenized it in the cryptic lysogen MS1138. The cryptic prophage in this strain contains the *cos* site at which the Ter function acts during DNA maturation (HERSKOWITZ and SIGNER 1970; MOUSET and THOMAS 1969), but has lost the right arm of the vegetative chromosome. Lysogenization was achieved by general recombination between the *b1319* genome and the cryptic prophage. Upon induction, the complex prophage will replicate without excision and maturation will occur by Ter action at the two *cos* sites. (See Figure 2c; see MATERIALS AND METHODS for details concerning the construction of this strain.) A phage carrying *chi3* alone was also synthesized for these experiments (see MATERIALS AND METHODS).

One-step growth curves for λ wild-type, λ*b1319* and λ*b1319chi3* are shown in Figure 6. The growth of λ*chi3* is indistinguishable from that of wild type. λ*b1319* has a greatly reduced burst size but *chi3* raises this to almost wild-type levels and also removes the slightly reduced rate of lysis observed for the deletion alone. In some experiments there was a four- to five-minute delay in lysis by both λ*b1319* and λ*b1319chi3*. We do not know why this was not reproducible, but it is evidently an effect of the deletion and is not influenced by *chi3*.

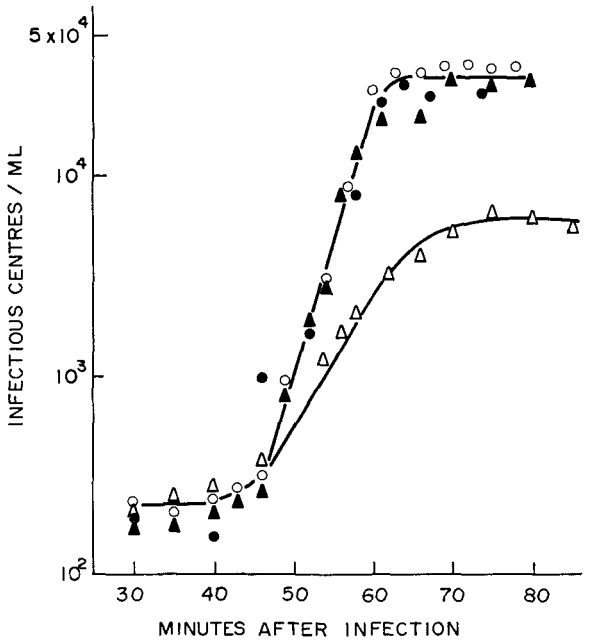


FIGURE 6.—One-step growth curves. C600 was grown to 2 × 10^8/ml in KM and infected at MOI = 0.1. After 10 minutes at 37° for adsorption, anti-lambda serum was added to give a final $k = 1$. Incubation was continued for a further 10 minutes and the infected cells diluted to give 10^3/ml and a further 50× to give 20/ml. These two cultures were aerated at 37° and samples withdrawn for plating at the times indicated. ○ = λ$^+$; ● = λ*chi3*; △ = λ*b1319*; ▲ = λ*b1319 chi 3*.

The results of our genetic experiments and those reported by LAM *et al.* (1974) and by MCMILIN, STAHL and STAHL (1974) suggested that the burst size increase produced in *b1319* by *chi3* should be Rec-dependent. As described in the introduction, Spi⁻ phage are also Fec⁻ and will not grow in a *recA* host due to the action of the RecBC nuclease. Burst sizes were therefore measured in *recArecB* and *recB* hosts, with an isogenic *rec*⁺ control. Results of these experiments are shown in Table 9. It can be seen that the burst sizes found in AB1157

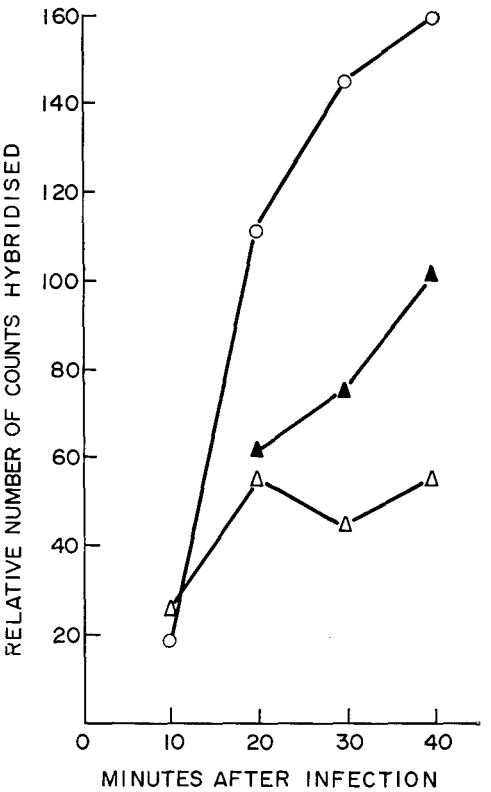


FIGURE 7.—DNA synthesis. CR34 Thy⁻ was grown to 2×10^8/ml in T broth supplemented with 0.2% maltose and 10 μg/ml thymidine and infected with phage at MOI = 5. At the times indicated, a 5 ml aliquot of the culture was transferred to a bubbler tube containing 10 μCi ^{3}H-me-thymidine (Schwarz-Mann) and aerated for three minutes. Incorporation was stopped by pouring into a centrifuge tube on ice containing unlabelled thymidine and NaCN to final concentrations of 200 μg/ml and 4 mM, respectively. Cells were lysed by 'method B' of YOUNG and SINSHEIMER (1967), with the exception that the lysozyme concentration was reduced 10-fold. The lysed cultures were heated for five minutes at 65° to complete lysis and reduce aggregation of the DNA (G. MOSIG, personal communication) and dialyzed extensively against SSC/10 at 4°. The DNA was denatured by sonication and boiling and hybridization was carried out on filters as described by DENHARDT (1966). 1 μg of unlabelled DNA per filter was used and hybridization was carried out with the labelled DNA diluted to a level below saturation. The number of counts per minute hybridized was averaged from duplicate measurements and normalized to 10^8 infected cells in the original culture. These results were then expressed relative to the value for λ*b1319 chi3* 40 minutes after infection arbitrarily set = 100. The figure shows average results from three independent experiments with each phage. ○ = λ+; △ = λ*b1319*; ▲ = λ*b1319 chi3*.

TABLE 9

Effect of the Rec system on burst size

Phage	Bacterial host		
	AB1157 (*rec*$^+$)	JC5495 (*recArecB*)	JC5743 (*recB*)
λ$^+$	108	73	110
λ*chi3*	76	56	74
λ*b1319*	17	22	18
λ*b1319chi3*	80	26	20

The burst sizes in AB1157 and JC5495 are the averages of duplicate determinations. The measurements in JC5743 were performed once only. Method was as described in Figure 6, except that unadsorbed phage were measured prior to addition of anti-serum and growth was in TB. Phage production was measured after addition of chloroform 60 min after infection and burst size expressed as pfu produced per pfu adsorbed.

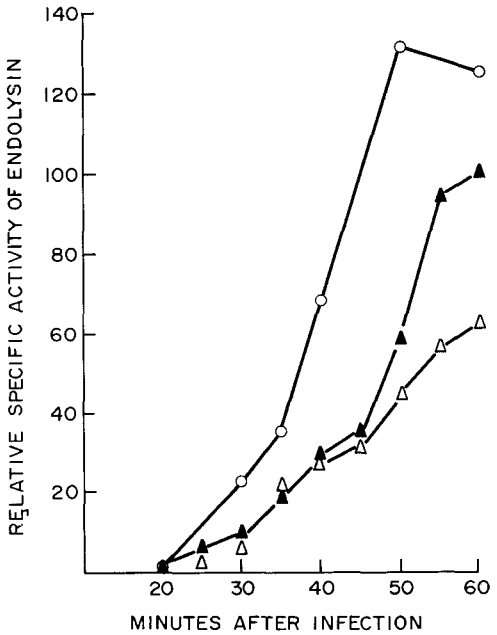


FIGURE 8.—Endolysin synthesis. The following procedure was adapted from published methods for endolysin assay and was found to improve reproducibility.

C600 was grown to 2 × 10^8/ml in KM at 37° and infected with phage at MOI = 5–10 at a final cell concentration of 10^8/ml. After aeration for 15 minutes, the infected cells were diluted tenfold into KG without Mg^{++} and aeration continued. At the times indicated, samples were removed from the cultures and lysed with chloroform in the presence of EDTA and dithiothreitol (DTT) at final concentrations of 10 mM and 1 mM, respectively. Samples were kept on ice and assayed as soon as possible. The EDTA removes residual Mg^{++}, which is inhibitory to the enzyme, and the DTT stabilizes the single —SH group of the enzyme (IMADA and TSUGITA 1971). It was found that C600 is preferable to other bacterial strains as host for these experiments, giving consistently higher and more reproducible enzyme activities.

The substrate for the enzyme assay, chloroform-washed C600 cells, was prepared as described by BLACK and HOGNESS (1969) except that the final preparation was lyophilized and stored at —20°. Other details of the assay are given by DAMBLY, COUTURIER and THOMAS (1968).

Specific activities were calculated per 10^7 killed cells. Results from five experiments were normalized to the specific activity of λ*b1319 chi3* at 60′ and averaged to produce the graph above. ○ = λ+; △ = λ*b1319*; ▲ = λ*b1319 chi3*.

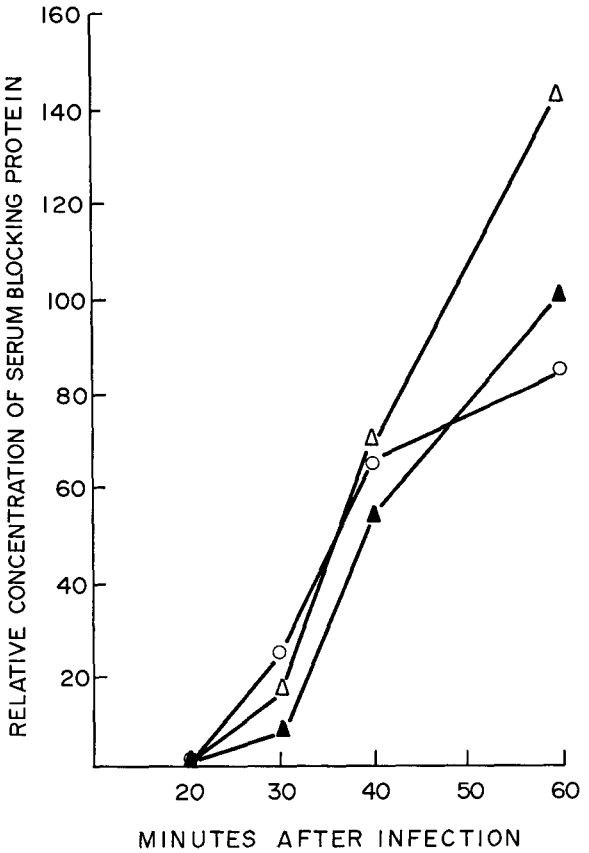


FIGURE 9.—Serum blocking protein synthesis. Serum blocking protein was measured as described by BUCHWALD and SIMINOVITCH (1969) using aliquots of the same cultures used for measurement of endolysin but lysed without the addition of EDTA and DTT (see legend to Figure 8).

Relative amounts of SBP were normalized to 10^7 killed cells. Results from three experiments were normalized to the SBP production by *b1319 chi3* at 60′ and averaged to produce the graph above. ○ = λ+; △ = λ*b1319*; ▲ = λ*b1319 chi3*.

(*rec*$^+$) follow the same pattern as in the one-step growth experiment in C600. In the *recArecB* and *recB* infections, however, the burst sizes of λ*b1319* and λ*b1319chi3* are the same. Thus the burst size increase produced by *chi3* is Rec-dependent. The relatively low burst size of both λ*b1319* and λ*b1319chi3* in the *rec*$^-$ hosts is presumably a consequence of their *red*$^-$ genotype (see ENQUIST and SKALKA 1973).

To further clarify our understanding of *chi* we have made measurements of DNA and late protein synthesis in infected cells.

DNA synthesis was measured by pulse-labelling infected cells with ^{3}H-thymidine. To measure the incorporation into phage DNA, samples extracted from infected cells were hybridized to unlabelled DNA from mature phage immobilized on filters (DENHARDT 1966). We find that *b1319* synthesizes consider-

TABLE 10

Phage and endolysin production after induction

Prophage	Burst size*	Endolysin*
λ+	43	171
λ*chi3*	40	171
λ*b1319*	5.3	71
λ*b1319 chi3*	9.7	(100)

* Lysogens of MS1138 were grown at 34° to 2 × 10^8/ml in KG, chilled on ice, collected by centrifugation and resuspended at 2 × 10^9/ml. The phage were induced by diluting 10× into KG without Mg^{++} at 42°, aerated for 10 min and then shifted to 37° for the remainder of the experiment. Endolysin was measured 60 min after induction as described in the legend to Figure 8. Burst size is calculated as pfu produced per killed cell. Results shown are averaged from seven experiments.

ably less DNA than wild type and that, while *chi3* enhances the rate, the final level attained is still below that of the wild-type control (Figure 7 and Table 11).

Two late gene products were measured: endolysin, the product of the *R* gene (DEL CAMPILLO-CAMPBELL and CAMPBELL 1965) and serum-blocking protein (SBP), the product of gene *J* (DOVE 1966; BUCHWALD and SIMINOVITCH 1969). The production of endolysin by λ*b1319* was found to be affected by *chi* to about the same extent as DNA synthesis (Figure 8, Table 11). The synthesis of SBP, on the other hand, showed a different pattern. λ*b1319* alone produced more SBP than wild type by 60 min after infection and *chi* reduced this to approximately wild-type levels (Figure 9).

We have also measured burst sizes and endolysin synthesis following induction of MS1138 lysogens of the appropriate phage. Results of these experiments are shown in Table 10 and are compared to the results from infection experiments in Table 11. The relative amounts of endolysin produced are essentially the same as following infection. We also find a *chi*-dependent burst size increase. but the effect is smaller than in infection.

TABLE 11

Comparison of effects of chi *on phage and macromolecular synthesis*

Phage	Burst size			Endolysin synthesis		DNA synthesis
	(a) Infection (i) Figure 6	(a) Infection (ii) Table 9	(b) Induction Table 10	(a) Infection Figure 8	(b) Induction Table 10	(Infection) Figure 7
λ+	6.7	6.4	8.1	2.0	2.4	2.9
λ*chi3*	5.4	4.5	7.5	—	2.4	—
λ*b1319*	(1)	(1)	(1)	(1)	(1)	(1)
λ*b1319chi3*	6.4	4.7	2.1	1.6	1.4	1.9

These figures are derived from the data presented in the Tables and Figures indicated normalized to the measurements for λ*b1319*.

DISCUSSION

The work we have presented deals with two related phenomena:
1) We have isolated a new set of deletion mutants of the central region of the lambda genome.
2) We have described a new class of presumed point mutants which enhance the reproductive capacity of the phage which carry the new deletions.

We shall begin by summarizing the conclusions for each of these separately.

1) Deletions

The first systematic study of deletions in a lambdoid phage was reported by FRANKLIN (1967). These mutants were isolated from a terminally deleted ϕ80-λ hybrid prophage and two of the deletions studied were shown to remove genes essential for vegetative recombination. An extensive analysis of deletion mutants of wild-type lambda has also been described previously (PARKINSON and HUSKEY 1971; PARKINSON 1971; DAVIS and PARKINSON 1971), but the mutants these authors studied did not include deletions of the *red* genes. It was suggested that the failure to find such mutants was due to their reduced burst size and the resulting counter-selection in the recycling procedure used to enrich EDTA-treated lysates for deletions (PARKISON and HUSKEY 1971).

In this paper, we have described the use of the positive selection afforded by the Spi$^-$ phenotype to avoid recycling in the isolation of phage deleted for all of the recombination genes of lambda. We have also described a procedure for the isolation of deletions without recycling by the use of plates containing EDTA to prevent plaque formation by phage phenotypically resistant to EDTA inactivation in liquid. This procedure allows the detection of deletions that would be selected against in recycling but for which there is no positive selection. By using this method, we have in fact been able to isolate *red*$^-$ (Spi$^+$) deletions (HENDERSON, unpublished experiments).

We have mapped several of the Spi$^-$ deletions by electron microscopy of heteroduplex DNA. Figure 4 shows the physical position of these deletions with respect to known genes in this part of the map. Consideration of the molecular weights of the proteins produced by these genes indicates that the *exo–cIII* region is saturated. The *exo*, *bet* and *gam* gene products have been purified and their molecular weights measured. (Exonuclease = 24,000; beta protein = 28,000 (RADDING *et al.* 1971), gamma protein = 16,000 (KARU, SAKAKI, ECHOLS and LINN, manuscript in preparation). The corresponding lengths of lambda DNA needed to encode these proteins are 0.0156, 0.0188 and 0.0104 respectively, assuming that 1% of the lambda genome is 465 nucleotide pairs (DAVIDSON and SZYBALSKI 1971).

The left end of *bet* must be to the right of 0.661 since this is the right endpoint of both the *bio69* substitution (HRADECNA and SZYBALSKI 1969) and the *b1451* deletion (this work). Both these phage are *exo*$^-$*bet*$^+$ (MANLY, SIGNER and RADDING 1969; this work). The *bet* and *gam* genes require a total of 0.0292 lambda DNA and thus the minimum position for the right end of *gam* is at 0.690. Since

the right ends of our deletions with endpoints in *cIII* span the region 0.689–0.692, it appears that *bet* and *gam are* the only proteins that can be accommodated between the end of *bio69* and the *cIII* gene, as defined by our deletions. It follows that only a small part of the *exo* gene can be present in *bio69* and *b1451* and that there cannot be another gene between *exo* and *bet*. *b1451* must remove almost the whole of *exo* and probably does not extend more than 50–100 nucleotides left of the gene.

Heteroduplex measurements of p*bio* and d*bio* substitution mutants lead to similar conclusions regarding saturation of this region (SZYBALSKI and SZYBALSKI, manuscript in preparation).

2) *chi* mutations

We have described the detection and partial characterization of mutations termed *chi* that suppress the poor growth phenotype of Spi$^-$ deletions. Our genetic experiments demonstrate that *chi* mutations can occur at several sites in the lambda genome. No non-homology is observed in DNA heteroduplexes of *chi*$^+$ and *chi* phage examined by electron microscopy, which rules out deletions or substitutions of more than about 50–100 nucleotides as the cause of *chi* mutations. The mutations can also be readily induced with 2-aminopurine (HENDERSON, unpublished experiments) and may therefore represent point mutations.

Attempts to separate *chi* from Spi$^-$ λp*bio*s failed, which implies that there is a *chi* within the *bio* DNA substitution of these phage. This is in agreement with the results of MCMILIN, STAHL and STAHL (1974) who showed that there is a recombination hot-spot in or close to the *bio* substitution. The λp*bio* phage appear to carry *chi* when isolated and these findings therefore suggest that *chi* sites occur within the *E. coli* chromosome at some points.

We have also shown that a *chi* mutation can arise within the *b1262* substitution, which is a segment of DNA of unknown function that presumably originated in the *E. coli* genome. Thus, the potential for formation of new *chi* mutations is probably not limited to the lambda genome.

3) Interaction of *chi* mutations with Spi$^-$ deletions

The new deletions have extended our knowledge of the interactions of phage and host functions related to the role of recombination in replication and maturation of phage DNA. The genes affecting generalized recombination in lambda have been thought of as 'non-essential' for phage growth due to the plaque-forming ability of phage with mutations in these genes. More recently, however, it has been shown that these mutants have abnormal aspects to their DNA replication (ENQUIST and SKALKA 1973; K. BARTA and J. ZISSLER, personal communication). The severe reduction in growth that we have found to be produced by the Spi$^-$ deletions confirms that the genes lost do indeed play a major role in the normal life cycle of the phage. As described below, the effects we have observed can be explained in terms of the known effect of a *red*$^-$*gam*$^-$ genotype on replication.

We have shown that the effect of the deletion can be circumvented by the introduction of a second, point, mutation called *chi* elsewhere in the genome. Such mutations are dependent on the host Rec system for their action and have been shown by LAM *et al.* (1974) to result in a stimulation of recombination in the vicinity of the mutation. Results of our genetic experiments also indicate effects of *chi* mutations on recombination.

We have shown that *chi* increases both the burst size and the amount of DNA synthesized by a Spi⁻ deletion. It is striking, however, that the increase in DNA synthesis is much smaller than the increase in burst size (see Table 11). These observations are consistent with the hypothesis put forward by LAM *et al.* (1974), that *chi*-suppression is the result of an increase in the amount of maturable DNA produced by recombination. This DNA is otherwise un-maturable, being trapped as monomeric circles due to the *red⁻gam⁻* genotype of the phage (ENQUIST and SKALKA 1973; see also beginning of paper). The *chi*-enhanced recombination could presumably occur with relatively little concomitant DNA synthesis since only 'repair' synthesis may be required to complete duplex recombinant molecules. We are unable to say if all of the observed increase in thymidine incorporation represents such repair or if in fact *chi* allows some molecules to replicate further, either as early 'theta forms' or late 'rolling circles.'

It is less clear how the results of the endolysin and serum blocking protein measurements should be interpreted. Any model should account for the following observations: Firstly, the *b1319* deletion results in a reduction of endolysin synthesis but an increased production of serum blocking protein. Secondly, *chi3* increases endolysin production by *b1319*, although the amount made is still less than following infection by wild-type phage, but the mutation reduces the amount of SBP produced to wild-type levels.

BUCHWALD and SIMINOVITCH (1969) have shown that the amount of SBP produced during infection by lambda is about fivefold in excess of that finally incorporated into phage particles. Further, they were able to demonstrate that SBP free in solution or on free tails is no more reactive than SBP in mature phage particles. These observations eliminate the possibility that the increased SBP activity observed in lysates of *λb1319*-infected cells results from an increase in free protein due to the smaller burst of mature phage.

A more complex situation exists in the case of the induced lysogens (Table 10 and Table 11). We expect that prophages containing *b1319* will replicate *in situ* after induction (see RESULTS for discussion of these prophages). There should therefore be few, if any, free monomeric genomes available as substrates for *chi*-enhanced concatemerization. In keeping with this expectation, we observe a relatively small effect of *chi* on burst size. The production of endolysin nevertheless shows almost as large an increase as observed after infection (see Table 11).

There are a number of mechanisms that might explain the influence of the deletions and *chi* mutations on protein synthesis. For example, the enhanced DNA synthesis produced by *chi* in a Spi⁻ deletion could result in gene dosage effects. Within this framework, however, we cannot adequately account for the

differential effects on SBP and endolysin production. These seem to indicate an unexpected level of complexity in the late gene control systems.

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Corresponding editor: H. ECHOLS