CHI-STIMULATED RECOMBINATION BETWEEN PHAGE λ AND THE PLASMID λdv

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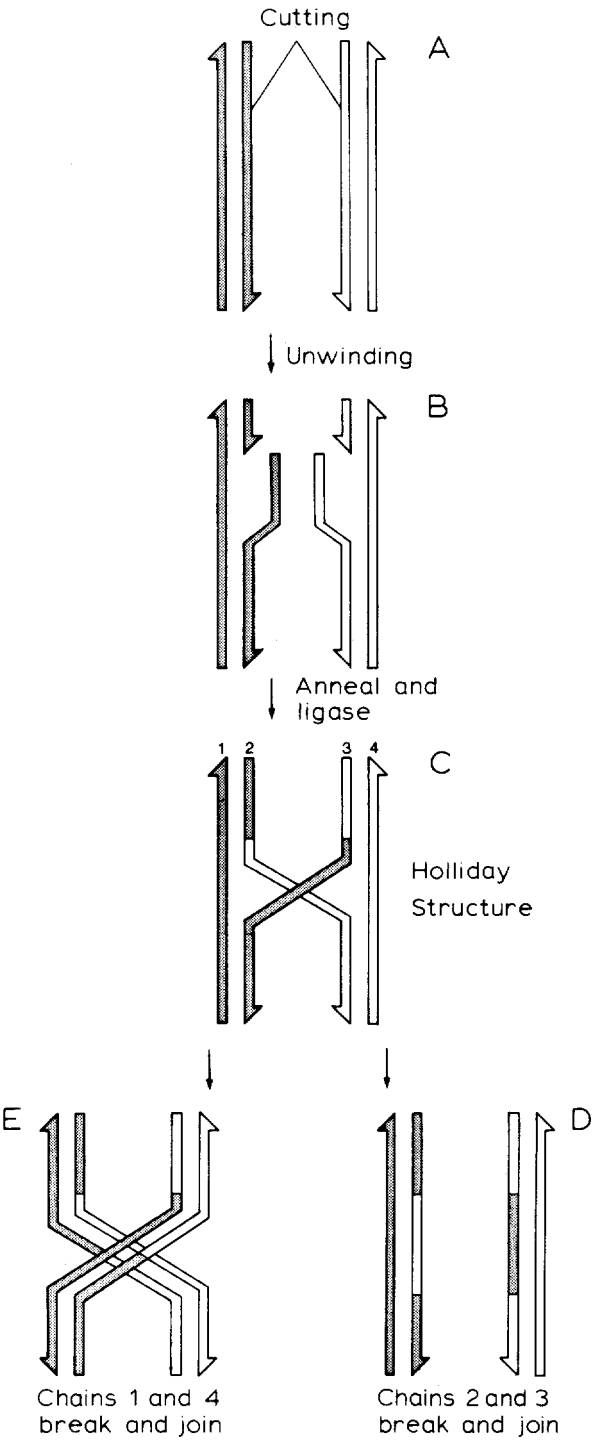
ABSTRACT

Chi promotes Rec-mediated recombination between phage λ DNA and the homologous plasmid λdv. In the absence of Chi, some of the interactions splice λdv into λ, whereas others patch information from λdv into λ. When Chi is in the phage DNA, splices and patches are increased in frequency by the same factor. This result strengthens the analogy between Chi and recombination-promoting elements in fungi. It also rules out one model for the previously reported orientation dependence of Chi phenotype.

EUKARYOTIC chromosomes that are genetically recombinant for close markers (e.g., those within one gene) are about as likely to be parental as they are to be recombinant for flanking markers (for references, see STAHL 1979a). To handle this feature of recombination the model of HOLLIDAY (1964) (diagrammed in Figure 1) postulates an intermediate ("Holliday structure") whose resolution can occur with equal probability in either of two ways. One resolution (Figure 1, bottom right) gives chromosomes that are mostly parental but have single chain "patches" from the other chromosome. The other resolution unites duplex segments from the two parents via "splices" (Figure 1, bottom left). Markers that are far apart compared with the length of a "splatch" (splice or patch) recombine primarily by the second route, with the entire splice falling in the interval between the markers. Markers close together compared with the length of a splatch, on the other hand, recombine by both routes. With splice and patch resolutions equally frequent, as presumed in Holliday's model, close marker recombinants are equally parental or recombinant for flanking markers with equal frequency.

Another feature of the model is that splice formation leads to reciprocal exchange for flanking markers. Recent studies in eukaryotes have forced modifications in Holliday's model (STAHL 1979a), but the central concept of an intermediate like that in Figure 1 has strong support. Such Holliday structures were seen in yeast (BELL and BYERS 1979) and in *E. coli* (POTTER and DRESSLER 1976; THOMPSON *et al.* 1975; THOMPSON, CAMIEN and WARNER 1976; BENBOW, ZUCCARELLI and SINSHEIMER 1975), and their relevance to recombination is established (IKEDA and KOBAYASHI 1979).

In fungi, the probability of recombination is conditioned by sites along the chromosome (MURRAY 1963; LISSOUBA *et al.* 1962; for further references see


Cutting
A
Unwinding
B
Anneal and
ligase
1
2
3
4
C
Holliday
Structure
E
D
Chains 1 and 4
break and join
Chains 2 and 3
break and join

STAHL 1979a), and models for recombination have focused on the presumed roles for these special sites called recombinators. In Holliday's model (Figure 1), the sites are the points at which chains of like polarity exchange to form the Holliday structure.

In *E. coli*, genetic elements that exalt exchange in their neighborhood have been demonstrated. These elements, Chi, reveal themselves when they are in phage λ whose genotype has been altered (Red^- Gam^-) so that recombination occurs via the Rec system (RecBC pathway) of *E. coli* (for review see STAHL 1979b). Chi enhances the production of splices as do the recombinators of fungi. The analogy between Chi and the recombinators of eukaryotes will be strengthened if Chi is shown to promote patch, as well as splice, formation and to promote macroscopically reciprocal exchange.

Efforts to demonstrate Chi-induced patches in a standard λ cross encounter some difficulties. In order for a Red^- Gam^- chromosome to be packaged *in vivo*, it must be dimerized by splicing. The splice that promotes the chromosome to packagable status will, of course, occur near the Chi. Even if the Chi-containing chromosome had enjoyed a patch in an earlier encounter, evidence of this patch is apt to be obscured by the obligatory splice.

In phage, the classical test of reciprocality of exchange involves the examination of virion yields from individual infected cells (HERSHEY and ROTMAN 1949; BRESCH 1955; WEIL 1969; SARTHY and MESELSON 1976). A stronger correlation in the numbers of complementary recombinants than of noncomplementary recombinants implies (some) reciprocality. Our efforts to assess reciprocality of Chi-mediated exchange in this way failed (F. W. STAHL and J. M. CRASEMANN, unpublished results).

It seemed possible that both of our aims could be met with a single experiment in which λ is crossed to a homologous plasmid. (1) Chi-stimulated incorporation of a plasmid into λ would provide evidence of reciprocality (the validity of this reasoning will be questioned in DISCUSSION). (2) If λ and the plasmid are genetically marked, then evidence regarding patch formation can be obtained as well. Those interactions that lead to splices will simultaneously incorporate the plasmid marker and the plasmid DNA into the λ genome with detectable genetic consequences and the detectable physical consequence of increased density of the virion. Those interactions that lead to patches, however, will not alter the virion density but will, sometimes, result in the incorporation of the plasmid marker.

The plasmid λdv021 is a circular version of part, *cI-P*, of λ's chromosome

FIGURE 1.—In Holliday's model, exchanges of the two complementary chains of a duplex are separated in time. Exchange between chains of one polarity (A, B) yields a stable intermediate (Holliday structure) (C). Resolution of that intermediate is by either of two routes. A second exchange occurring between the two chains which originally exchanged to form the Holliday structure yields chromosomes that have patches from each other but are not exchanged for flanking markers (D). Exchange between the other two chains, however, produces chromosomes in which duplex segments from the two parents are held together at splices (E). When recombination occurs between circles, as in λ, only the latter route unites the circles into a dimer.

(Figure 2). A phage, that is either O^- or P^- can become O^+ or P^+, respectively, by exchange with the plasmid. If the superinfecting phage chromosome is short enough, it can become O^+ or P^+ either by picking up a patch from λdv or by splicing the entire λdv into itself (BERG 1974). Neither of these events qualifies the phage chromosome for packaging; on the contrary, each O^+/P^+ phage chromosome must splice with another phage chromosome, which will, in general, be O^-/P^-. We may reasonably assume that each class of O^+/P^+ chromosome is equally likely to enter into a subsequent phage by phage splice that will lead to packaging. The packaged O^+/P^+ phage can then be sorted out into λ × λdv splices *vs.* λ × λdv patches according to density. To ascertain whether Chi promotes patch and splice formation by the same factor, the relative amounts of O^+/P^+ phage of distinguishing densities can be compared for χ^0- and for χ^+-infecting phage.

A brief report of a part of this work has appeared (STAHL *et al.* 1980a).

MATERIALS AND METHODS

Phage mutations are listed in Table 1. Their map positions are shown in Figure 2. Making of *cos*-aberrant λs is described elsewhere (KOBAYASHI *et al.* 1982; M. FEISS, I. KOBAYASHI, W. WIDNER, unpublished results). In Figure 2, the natural *cos* is shown as a rightward arrow. This primary *cos* can be wild type (*cos*$^+$) or can be inactivated by a deletion mutation, *cos2* (Table 1), which is shown as an asterisk in Figure 8. The arrow in the middle of the map (Figure 2) symbolizes another *cos* sequence obtained by molecular cloning. The secondary *cos* is oriented leftward in the initial clone. We turned it rightward by inverting the *Eco*RI·B fragment.

Bacterial strains are described in Table 2.

Phage methodology was standard (see HERSHEY 1971).

Quantitative gel electrophoresis: To determine the relative amounts of intracellular monomer and dimer λdv, a cleared lysate (from the culture of JC9937 (λdv) used to obtain the data in Figure 7) was prepared and phenol extracted. The plasmid DNA was electrophoresed on a 1% agarose vertical slab gel in 40 mM Tris-acetate, 5 mM Na acetate, 1 mM EDTA for 16 hr at 4 V/cm. Gels were stained for 1 hr in electrophoresis buffer containing 2 μg/ml ethidium bromide and were photo-

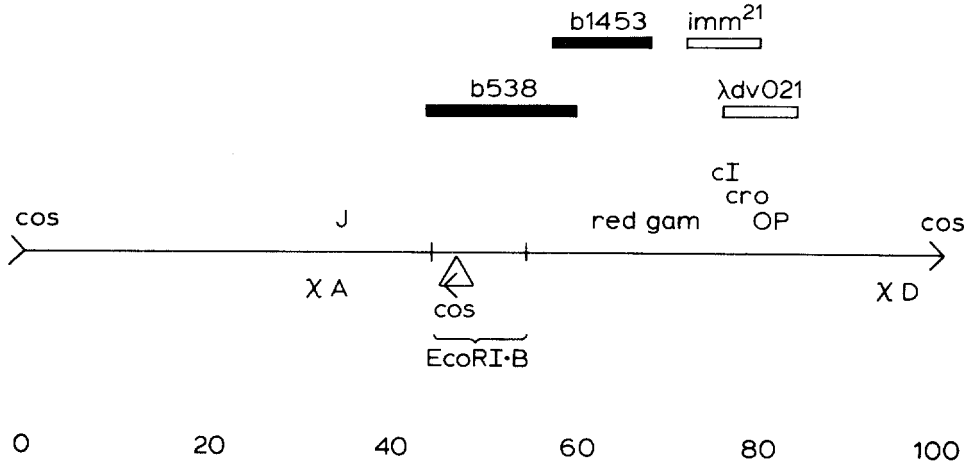


FIGURE 2.—Map of λ showing the features referred to. The scale is percentage of distance from the left to the right end.

TABLE 1

Mutations of λ employed

Mutation	Properties	Reference
λdv021	Plasmid derivative of λ bearing genes *O* and *P*	BERG 1974
imm^{21}	Immunity region of phage 21 substituted into λ	CAMPBELL 1971
b1453	Red$^-$ Gam$^-$ deletion	HENDERSON and WEIL 1975
sus029	Amber mutation in gene *O*	CAMPBELL 1961
susP80	Amber mutation in gene *P*	CAMPBELL 1961
χD$^+$123	Active Chi site to right of genes *O* and *P*	STAHL, CRASEMANN and STAHL 1975
b538	Red$^+$ Gam$^+$ deletion	DAVIS and PARKINSON 1971
gam210	Amber mutation in *gam*	ZISSLER, SIGNER and SCHAEFER 1971
cos2	22 base pair deletion removing *cos*	I. KOBAYASHI *et al.* 1982

TABLE 2

Strains of Escherichia coli *employed*

Strain	Properties	Source/reference
JC9937	Su$^-$ *rec*$^+$	A. J. CLARK, Berkeley
JC11,810	Su$^-$ *recF143*	A. J. CLARK, Berkeley
JC9935	Su$^-$ *recA13*	A. J. CLARK, Berkeley
JM1	SuIII$^+$ *recB21 sbcA20*	STAHL *et al.* 1980b
JC9387	Su$^-$ *recB21 recC22 sbcB15*	STAHL and STAHL 1977
RM200	Su$^-$ *recA1 recB21*	STAHL, STAHL and MALONE 1978

graphed under UV light using Polaroid type 55 film. Negatives were traced using an LKB microdensitometer. Relative concentrations of monomer and dimer λdv were determined from samples that fell in the linear response portion of the film.

RESULTS

Infection of E. coli rec$^+$ *(λdv) by* λsusO, *Chi*$^+$ *or Chi*$^-$

λdv (Table 1) was established in *E. coli* strain JC9937 (Table 2) by the method of KELLENBERGER-GUJER, BOY DE LA TOUR and BERG (1974). A single culture grown from a single-cell isolate of *E. coli* (λdv) was divided, and the two portions were infected by λ*b1453 imm*^{21} *sus029* which was χD^0 or χD$^+$, respectively. χD, which is located to the right of the *cI-P* interval, acts leftward (STAHL *et al.* 1980b). Despite the fact that χD is outside the region of λs homology with λdv, it can be expected to stimulate λ × λdv recombination (STAHL and STAHL 1975). The *imm*^{21} substitution permits the phage to grow well in the presence of λdv (KUMAR and SZYBALSKI 1970; MATSUBARA 1972; BERG and KELLENBERGER-GUJER 1974) while the *b1453* deletion serves two roles: it makes the phage Red$^-$ Gam$^-$, allowing full expression of Chi phenotype, and it provides room in the chromosome for splicing in as many as three copies of λdv. Each lysate from these infections was centrifuged to equilibrium in a cesium formate gradient. Fractions were collected from the bottom and assayed on *recB* bacterial hosts, which give large plaques of Red$^-$Gam$^-$λ. Total phage were counted on a Su$^+$

strain, whereas Sus^+ phage were counted on a Su^- strain. The data obtained (Figure 3) show the following: (1) The yield of phage is five times as much in the Chi^+ (panel b) as in the Chi^- (panel a) infection. Such an enhancement of yield is characteristic of Chi action (MALONE and CHATTORAJ 1975). (2) The yield of phage into which one copy of λdv has been spliced is about 70 times as high for the Chi^+ as for the Chi^- infection, so the fraction of phage bearing λdv is about 14 times as high for the Chi^+ as for the Chi^- infection. Thus, by the criterion of circle incorporation (see DISCUSSION), Chi can promote reciprocal exchange. (3) In each experiment there are about one-fourth as many Sus^+ phage not carrying λdv as there are phage carrying one λdv. If these particles are interpreted as arising from patches, then Chi stimulates patch formation by the same factor that it stimulates splices. In a later section and in DISCUSSION we shall question the legitimacy of this assumption.

Infections using another host and another marker

The experiment was repeated and was also varied in two respects: (1) the *recF* strain JC11,810 (λdv) was used, and (2) a *susP* mutation was used instead of *susO*. The *recF* experiments were motivated by the possibility that elimination of the RecF pathway, which is not Chi stimulated (STAHL and STAHL 1977),

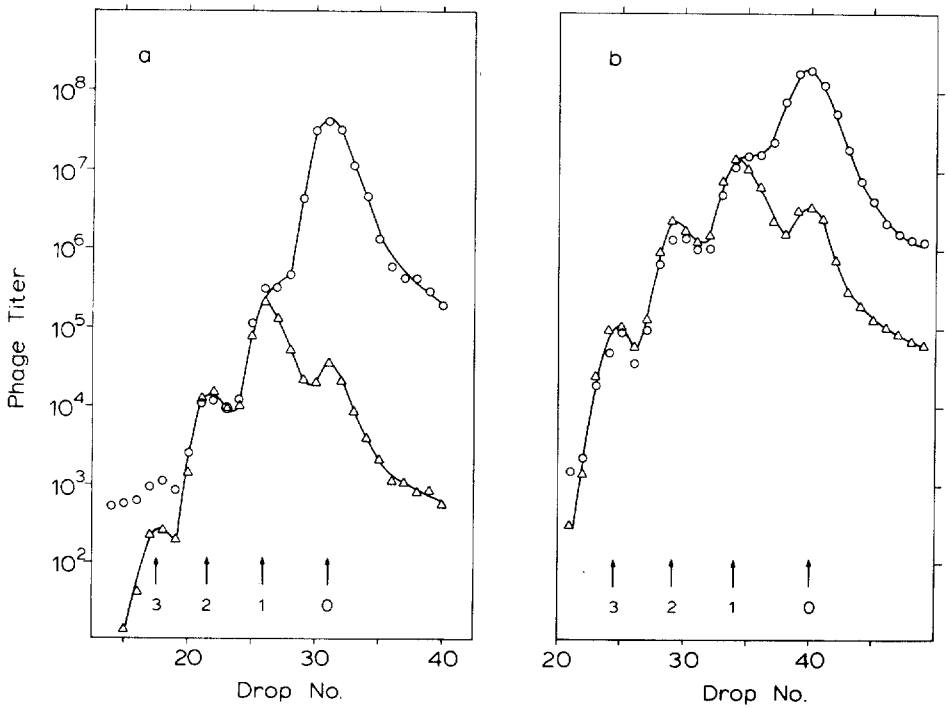


FIGURE 3.—Products of recombination between Red^- Gam^- λ and λdv in a rec^+ host. *E. coli* strain JC9937 (λdv) was infected with λ *b1453 imm*^{21} *sus029* (a) and with a χD^+ derivative of the same phage (b). The resulting lysates were centrifuged in a cesium formate gradient, and fractions were assayed on JM1 for total phage (○) and on JC9387 for Sus^+ phage (△). Density increases leftward, and peaks corresponding to λ carrying three, two, one or zero λdv's are indicated.

might enhance the Chi effects being examined. The *susP* experiment was performed to generalize the results obtained with *susO*. The results of these infections were essentially identical with those reported previously; all of the results are summarized in Table 3.

χA^+ does not promote recombination between λ and λdv021

It is a characteristic of Chi that it stimulates recombination in its own locale. Our conclusion that χD^+ in λ promotes exchange between λ and λdv would be strengthened by a demonstration that a Chi element far from the region of homology between λ and λdv does not stimulate that interaction. Figure 4 gives the results of such an experiment using χA, located to the left of the λdv region, in or near *J* (STAHL, CRASEMANN and STAHL 1975). Since this Chi site, like the others in λ, acts primarily to its left (STAHL *et al.* 1980b), it should not affect recombination with λdv. The expectation is supported by the data.

Chi-stimulated exchanges between λ and λdv are RecA dependent

Chi-stimulated λ × λ splice formation has been shown to be RecA dependent (STAHL and STAHL 1977; GILLEN and CLARK 1974). Is patch formation by Chi similarly specific to the Rec system? A requirement for RecA could be tested by establishing λdv in a *recA* host and using *red*$^+$ phages with and without Chi. As shown in Figure 5, neither splices nor patches are stimulated by χD^+ in the absence of RecA (and presence of λ's recombination functions, Red).

Sus$^+$ phages of ordinary density are not segregation products of chromosomes that have previously incorporated λdv

Several reconstruction experiments demonstrate that the rate at which our phage carrying λdv break down yielding Sus$^+$ phage of ordinary density is too low to contribute importantly.

Rate of breakdown of single-plaque isolates of Sus$^+$ λ carrying λdv: From experiments like that in Figure 3, three individual Sus$^+$ Chi$^+$ and three Sus$^+$ Chi$^-$ plaques on JC9387 were picked from the peaks corresponding to λ carrying a single λdv. A plate lysate from each was prepared on a *recA recB* host. These lysates contained less than 1% of particles without the λdv insert. Each of the lysates was grown for one cycle on a *rec*$^+$ host. Sus$^+$ segregants containing no duplication appeared as a minor peak on the light side of the major Sus$^+$ peak formed in a cesium gradient. The height of that peak relative to the major Sus$^+$ peak gives an estimate of the frequency of such Sus$^+$ segregants. We did not correct the minor peaks for contributions from the nearby, major peak, so these estimates exaggerate the rate of segregation. The three independent χ^0 lysates gave light peak to major peak ratios of such Sus$^+$ phages of 0.02, 0.02 and 0.03, respectively. The χ^+ preparations gave 0.02, 0.03 and 0.04, respectively. For one χ^0 and one χ^+ lysate, the *rec*$^+$ cultures were co-infected with *λb1453 imm^{21} sus029*, Chi$^-$ or Chi$^+$ as appropriate, to mimic more precisely the conditions of the original experiment. Light Sus$^+$ segregants were 0.02 of the major peak Sus$^+$ progeny in the Chi$^-$ reconstruction and 0.07 in the Chi$^+$ one. A similar experiment with co-infecting *λb1453 imm^{21} sus029* performed in the Su$^+$ host AB1157 gave ratios of 0.01 and 0.05 for the χ^0 and χ^+ phages, respectively.

TABLE 3

Chi stimulation of patches and splices

Experiment host number	Phage	Phage yield χ^+/χ^0	λ(λdv) yield χ^+/χ^0	Patch/splice χ^0	Patch/splice χ^+
JC9937 (λdv)	*susO*	5	70	0.18	0.25
JC9937 (λdv)	*susO*	20	86	0.16	0.21
JC11,850 (λdv)	susO	26	566	0.19	0.24
JC11,850 (λdv)	*susP*	8	86	0.20	0.31

Infections of bacteria carrying λdv were made by either *susO* or *susP* mutants of λ at a multiplicity of 7. The phage produced were centrifuged in cesium formate and fractions assayed with results like those shown in Figure 3. Phage yields were taken as titers of peak fractions assayed on *E. coli* strain JM1. Unadsorbed phage made a negligible contribution to the titer of the major λ peak. The yield of λ(λdv) is the titer on JC9387 in the peaks corresponding to the insertion of one λdv into the phage. This same titer is used as the denominator in the calculation of patch/splice. The numerator of that fraction is taken as the titer of phage of normal density that makes plaques on JC9387. The first line summarizes the data from Figure 3.

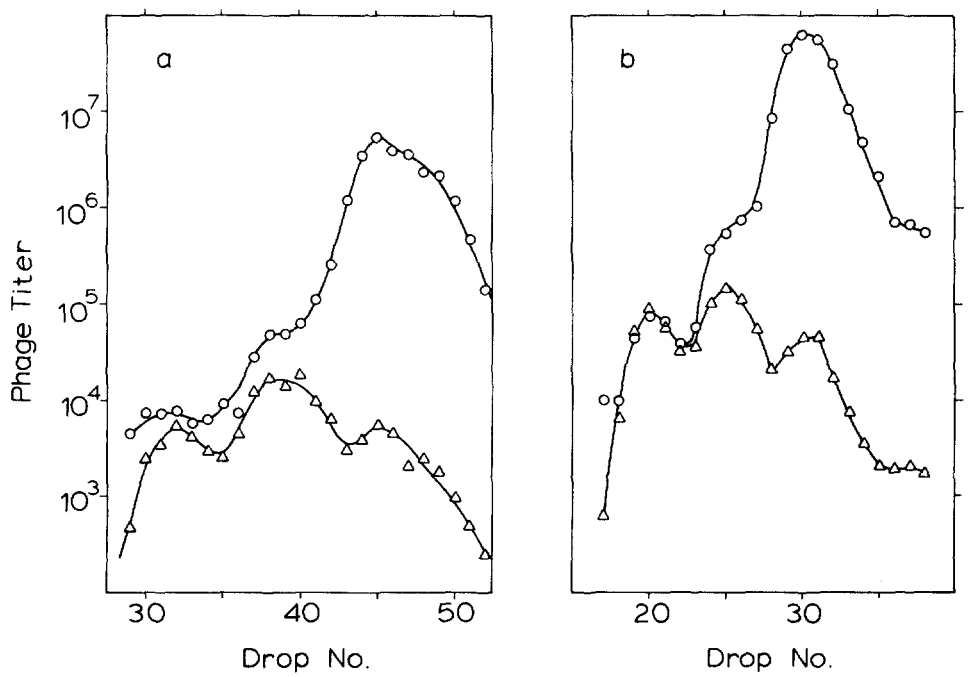


FIGURE 4.—Recombination between λ χA^+ and λdv. The *rec*$^+$ strain JC9937 (λdv) (isolate c1) was infected by λ *b1453 imm*^{21} *sus029* either χ^0 (a) or χA^+ (b). The resulting lysates were analyzed as in Figure 3.

Rate of segregation in a lysate made from a large number of λ(λdv): The rate of segregation of light Sus$^+$ phage from a given isolate of λ carrying λdv probably depends on the site of the splice that incorporated the λdv into the λ chromosome, either to the left or to the right of the *O* mutation. Thus, the individual isolates studied might give a misleading estimate of the average rate of segre-

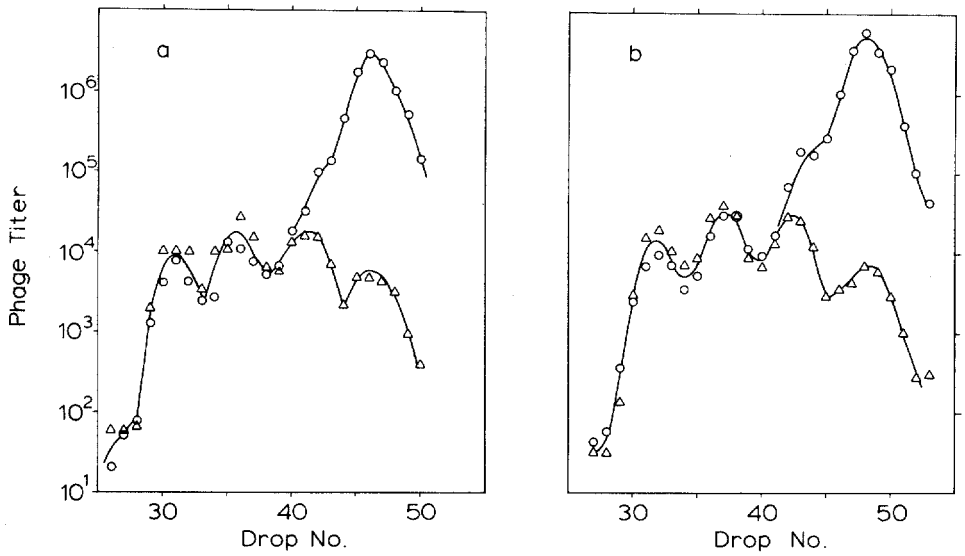


FIGURE 5.—Products of recombination between Red$^+$ Gam$^+$ λ and λdv in the *recA* host. *E. coli* strain JC9935 (λdv) was infected with λ *b538 imm*^{21} *sus029* (a) and with a χD$^+$ derivative of the same phage (b). The resulting lysates were analyzed as in Figure 3.

gation. From the major peaks of experiments like that in Figure 3, we made χ^0 and χ^+ plate lysates by direct inoculation with samples of the peaks (about 10^5 particles) on *recA recB* plating bacteria. The harvested stocks, whose frequencies of nonduplication phage were less than 1%, were then passed for one cycle of growth through JC9937. The light to major peak ratios were 0.02 and 0.05 for the χ^0 and χ^+ phages, respectively.

The data described reveal that λ*susO* segregates light Sus$^+$ phage in one cycle of growth in amounts far less than those seen in the experiments that reveal patches. Thus, the λSus$^+$ seen there can be attributed to a single event, *i.e.*, to a patch.

The results of these reconstructions show that the segregation from λdv-carrying phage is slow, in agreement with results of BERG (1971). They show also that Chi$^+$ λ carrying λdv segregate light Sus$^+$ phage faster than do Chi$^-$ ones. (The slight excess in the patch to splice ratio of the Chi$^+$ as compared with the Chi$^-$ experiments seen in Table 3 is attributable to this excess rate of segregation from the Chi$^+$ λ.) This result, not unexpected, supports further the conclusion that the peaks of Sus$^+$ phage of ordinary density (as in Figure 3) are primary products of interaction between λ and λdv.

The similarity in relative heights of the two peaks in the presence and absence of Chi is in itself an argument for the predominantly one-step origin of the lightest Sus$^+$ phage. If the light Sus$^+$ peaks were primarily the result of successive splices, then the Chi$^+$ peak would be far higher relative to its λ(λdv) peak than would the Chi$^-$ one.

The origin of λ *carrying two* λ*dv:* The existence of virions containing two λdv appears to undermine the aforementioned arguments by suggesting a high rate of complex events. For instance, the peak of phage with two λdv could come

about in two steps: (1) one λdv could be incorporated, then (2), after replication of that λ chromosome, unequal sister exchange could lead to a chromosome with two λdv (*i.e.*, a triplication of the *cI* region). Such an act would generate phage of ordinary (*i.e.*, no λdv) density, as well. Despite the results of our reconstructions, the similarity in heights of the Sus^+-two-dv and the Sus^+-no-dv peaks suggests that we should address such a possibility. The following experiment argues against a two-step origin for λ carrying two λdv.

An experiment like that in Figure 3 was conducted on an aliquot of a 1-liter culture. The bulk of the culture was used as a source for DNA that could be assayed for the relative amounts of monomer and dimer λdv. The data, in Figure 6, reveal that the uninfected culture contained monomers and dimers in the molar ratio of 8.5:1. This ratio is reproduced in the ratio of virions that picked up one or two λdv, respectively (Figure 7). Thus, phage with two λdv are primarily a result of a single exchange (between λ and a free λdv dimer). We conclude that Sus^+ phage bearing no λdv are likewise the result of simple interactions.

Chi in its "inactive" orientation does not stimulate patches preferentially

FAULDS *et al.* (1979) and YAGIL *et al.* (1980) showed that Chi's are fully active

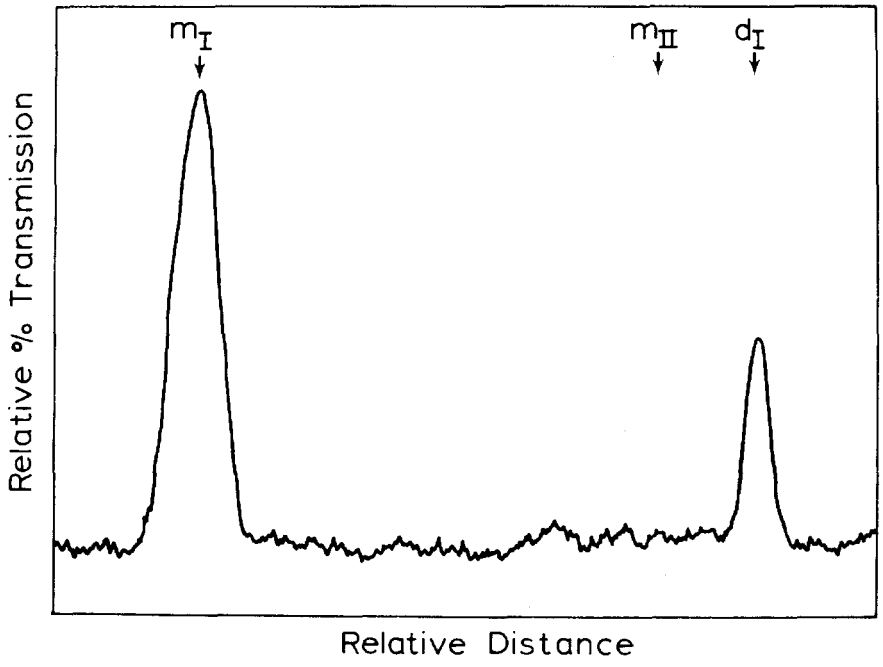


FIGURE 6.—Relative frequency of monomers and dimers in λdv population. Plasmid DNA was extracted from the culture of JC9937 (λdv) used to obtain the data in Figure 7. The DNA bands resulting from gel electrophoresis were photographed, and the intensities of the negative images were quantitated by densitometry as described in MATERIALS AND METHODS. The resulting trace showed the amount of DNA in the dimer band (d_I) to be 24% as much as in the monomer band (m_I) implying a molar ratio of monomer to dimer of 8.5:1. No nicked monomer (m_{II}) was seen in this sample. Electrophoresis was from right to left.

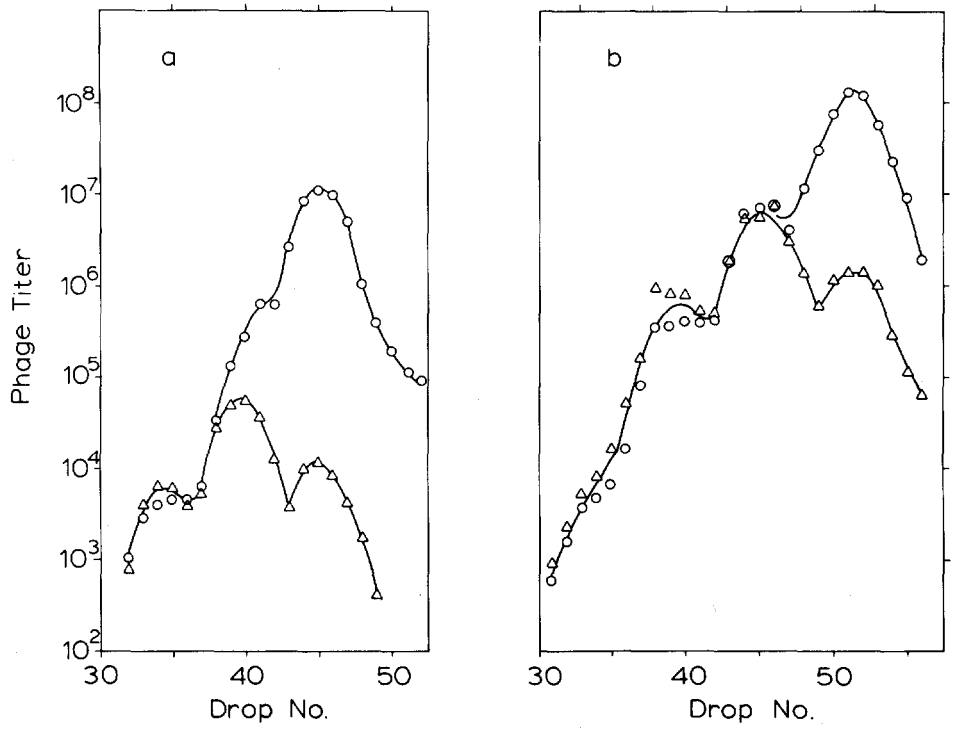


FIGURE 7.—Virion yields from infection of the culture whose λdv population is characterized in Figure 6. Infection was by the same pair of phage used in Figure 3. a, χ^0; b, χD^+. Analysis of lysates was conducted as in Figure 3.

in only one of their two possible orientations in the λ genome. Our demonstration that χD^+ stimulates patch and splice formation by the same factor bears on models for this orientation dependence of Chi activity. One model (FAULDS *et al.* 1979) proposed that Chi is but one of two kinds of elements that interact to effect exchange in λ. One of the kinds of elements is supposed to promote formation of Holliday structures, whereas the other kind promotes their resolution. When the two kinds of elements act on different chains, the resulting product is a splice; when they operate on the same chain, patches result (Figure 1). The model is complete if we then suppose (1) that Chi affects one of λs chains when in one orientation and the other chain when in the other orientation and (2) that the other element (not Chi) happens to be so disposed in λ as to affect only one of λs two chains. This model predicts that Chi in one of its orientations (the "active" one) promotes only splice formation with its attendant exchange of flanking markers and increase in viral yield, whereas Chi in its "cryptic" orientation promotes only patches. Our demonstration herein that a given Chi in a fixed orientation (the "active" orientation) promotes patches and splices by the same factor argues against the model. A second test that will be described examines its "inactive" orientation for preferential stimulation of patches.

Elsewhere (KOBAYASHI *et al.* 1982) we show that the orientation-dependent

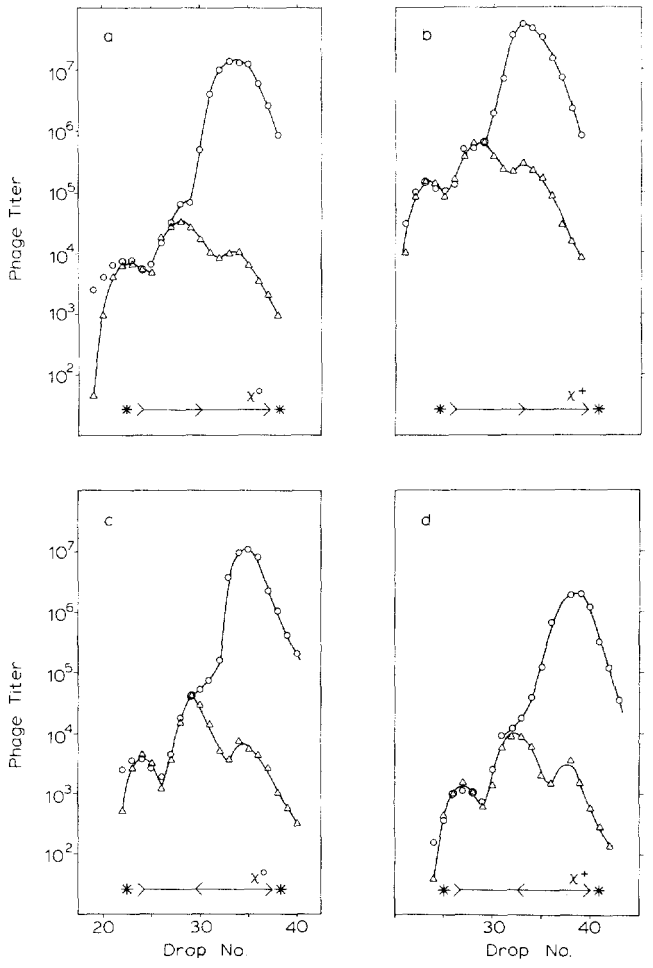


FIGURE 8.—Products of recombination between λdv and λ χD^+ whose activity is reduced by inversion of *cos*. The rec^+ strain JC9937 (λdv) was infected with λ *b1453 imm*^{21} *susP80*, in which the standard *cos* was inactivated by mutation (*cos2*) and a cloned *cos* was present in standard or inverse orientation. The resulting lysates were centrifuged and analyzed as in Figure 3.

Panel	Cloned cos	Chi	Chi phenotype
a	standard	χ^0	Chi^-
b	standard	χD^+	Chi^+
c	inverted	χ^0	Chi^-
d	inverted	χD^+	Chi^-

In this experiment only, the Sus^+ curves have been adjusted up or down to make these heaviest peaks coincide with the heaviest total phage peaks. Such adjustments, necessitated by variations in efficiency of plating, never exceeded a factor of two.

phenotype of Chi is dependent upon the orientation of *cos*, the site at which λ concatamers are cut during encapsidation. In the model for orientation dependence described, *cos* can play the role of the element that interacts with Chi to give a final outcome of a splice or a patch, respectively. If the model were

correct, then Chi rendered (relatively) inactive by virtue of a *cos* inversion would show preferential stimulation of patches over splices. To test this possibility, we used λ carrying a supernumerary *cos* "cloned" near the middle of the chromosome. The standard *cos* in this λ was inactivated by the *cos2* deletion, and we examined the effect of Chi when the cloned *cos* was in the standard or in the inverse orientation. The results (Figure 8) show that Chi rendered less active by inversion of *cos* does not manifest preferential stimulation of patches over splices.

DISCUSSION

Our primary conclusions are two: (1) Chi stimulates patches as well as splices, and (2) Chi stimulates reciprocal exchange. Both conclusions, however, must be qualified. (1) It must be kept in mind that the concept of a patch, diagrammed in Figure 1, is an abstraction. There is no way, at least at present, in any system, to distinguish a patch from a close pair of splices occurring simultaneously (STAHL 1979a). (2) The incorporation of circular λdv into λ is obviously interpretable as being due to reciprocal exchange (CAMPBELL 1962). However, our experiments do not rule out more complex possibilities, such as a succession of nonreciprocal events leading to the observed incorporation.

Previously (STAHL *et al.* 1980b), we reported a nonreciprocal aspect of Chi-mediated exchange. When Chi in one parent in a λ × λ replication-blocked cross stimulates exchange, the recombinant that inherits Chi is recovered as a virion less often than is its complement. This result implies a structural asymmetry in the circular dimer resulting from the exchange. The asymmetry may be temporary, however, and, when packaging does not intrude, may not interfere with the ultimate macroscopic reciprocality of the event.

Our results allow us to reject a model for the orientation dependence of Chi activity that proposes that Chi in one orientation makes only splices, whereas in the other orientation it makes only patches. The conditions under which we have demonstrated Chi-stimulated patches put a restriction on possible models for the mechanism of Chi action. In our experiments, only one of the two interacting chromosomes, λ and λdv, was χ^+. Since the patches were detected in λ, the chromosome that was χ^+, we can reject models in which Chi donates but does not accept patches. Whether or not Chi accepts patches preferentially to donating them, as does *cog* in Neurospora (CATCHESIDE 1974), must be addressed by further work.

DOUG BERG instructed us in the arts of λdv and provided us with strains. The work was supported by National Science Foundation grant PCM 8109799 and National Institutes of Health grant GM 20373.

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