Further Tests of a Recombination Model in Which χ Removes the RecD Subunit From the RecBCD Enzyme of *Escherichia coli*

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ABSTRACT

When one of two infecting λ phage types in a replication-blocked cross is χ^+ and DNA packaging is divorced from the RecBCD-χ interaction, complementary χ-stimulated recombinants are recovered equally in mass lysates only if the χ^+ parent is in excess in the infecting parental mixture. Otherwise, the χ^0 recombinant is recovered in excess. This observation implies that, along with the χ^0 chromosome, two χ^+ parent chromosomes are involved in the formation of each χ^+ recombinant. The trimolecular nature of χ^+-stimulated recombination is manifest in recombination between λ and a plasmid. When λ recombines with a plasmid via the RecBCD pathway, the resulting chromosome has an enhanced probability of undergoing λ × λ recombination in the interval into which the plasmid was incorporated. These two observations support a model in which DNA is degraded by ExoV from *cos*, the sequence that determines the end of packaged λ DNA and acts as point of entry for RecBCD enzyme, to χ, the DNA sequence that stimulates the RecBCD enzyme to effect recombination. The model supposes that χ acts by ejecting the RecD subunit from the RecBCD enzyme with two consequences. (1) ExoV activity is blocked leaving a highly recombinagenic, frayed duplex end near χ, and (2) as the enzyme stripped of the RecD subunit travels beyond χ it is competent to catalyze reciprocal recombination.

χ (5′-GCTGGTGG-3′) locally stimulates recombination mediated by *Escherichia coli*'s RecBCD pathway (STAHL, CRASEMANN and STAHL 1975; SMITH *et al.* 1981). When present in phage λ, χ stimulates recombinant to its left (on λ's standard map). The ability of χ to stimulate recombination when present in only one of two infecting λ parents in a lytic cycle phage cross allows us to question the reciprocality of χ-stimulated recombination by the examination of the pooled progeny from many infected cells ("mass lysates"). These investigations showed that the recombinant that inherits χ^+ is found less often than the one that does not (STAHL *et al.* 1980). Subsequent investigation (KOBAYASHI *et al.* 1984) appeared to demonstrate that most or all of the nonreciprocality in χ-stimulated exchange is due to preferential packaging of the newly arising χ^0 recombinant. This preferential packaging is intimately related to the mechanism by which the RecBCD enzyme finds the χ sequence.

To effect recombination, RecBCD enzyme enters a DNA duplex at a double chain break (STAHL *et al.* 1983). In the ordinary course of events, this break arises late in the lytic cycle when λ's terminase binds to the right of *cos* and cuts *cos*, yielding a linear λ chromosome with terminase bound at the left end (Figure 1) (KOBAYASHI, STAHL, and STAHL 1984). RecBCD enzyme enters the right end and travels leftward (STAHL *et al.* 1986) through the duplex, catalyzing recombination with only a low probability until it encounters a correctly oriented χ sequence. The encounter activates the enzyme, and exchange with a circular homologue occurs with heightened probability that decreases with distance to the left. As a result of the exchange, the chromosome is now part of a dimer, meeting a presumed prerequisite for packaging (*e.g.*, STAHL *et al.* 1972). The terminase (and a prohead that has bound to it) can package DNA extending from the *cos* that was cut to the *cos* of the homologue. The complementary recombinant is no longer part of a dimer and is, consequently, not packageable *per se* (reviewed by FEISS and BECKER 1983).

In Figure 1, the observed nonreciprocality is ascribed totally to this preferential packaging. Since the complementary recombinant does show some χ-stimulation, we must account for its occasional recovery in a phage particle. In the scheme of Figure 1, the neglected recombinant can appear among the progeny particles if it manages to dimerize by a subsequent, independent exchange. Alternatively, the indicated packaging event may sometimes fail and *cos* may reclose. If terminase then binds to *cos* on the χ^0 parent, the other recombinant can package from the dimer.

The scheme in Figure 1 was tested by KOBAYASHI *et al.* (1984) with $\chi^+ \times \chi^0$ crosses in which the χ^0 parent was deleted (*cos2*) for the cut site of *cos* (Figure 2). Each parent carried a cloned *cos* (*cosML*) in the middle of the standard map. These cloned *cos* sites

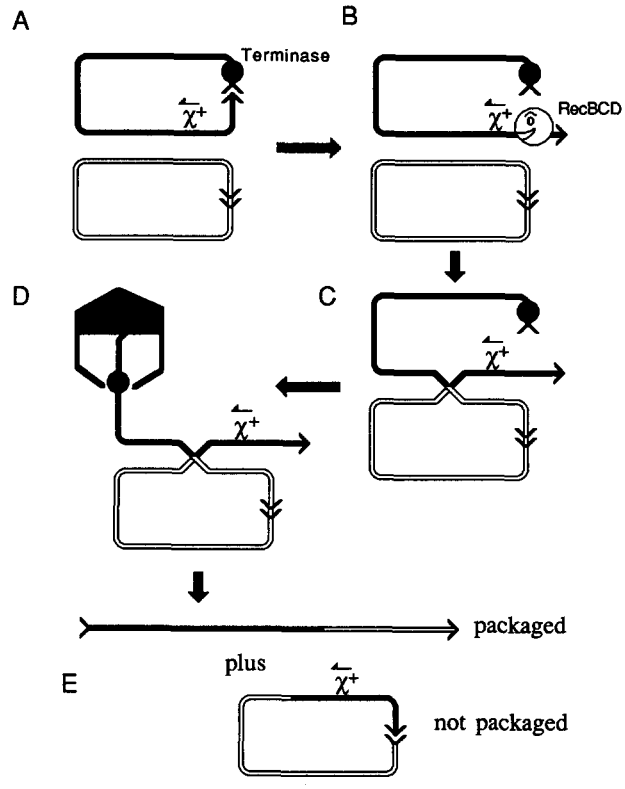

FIGURE 1.—*cos*-cutting by terminase initiates both recombination and packaging leading to the preferential recovery of one crossover product (KOBAYASHI *et al.* 1984). A, terminase binds to the "right" of *cos* and cuts *cos*. B, RecBCD enzyme enters at the right end of λ and travels leftward until it is activated by χ. C, The enzyme catalyzes a reciprocal exchange. D, terminase binds a prohead and packages the recombinant that does not inherit χ^+. The remaining recombinant, containing χ^+, can be packaged if it succeeds in dimerizing by recombination with another phage. When recombinant particles are enumerated, recombination appears to have been somewhat nonreciprocal.

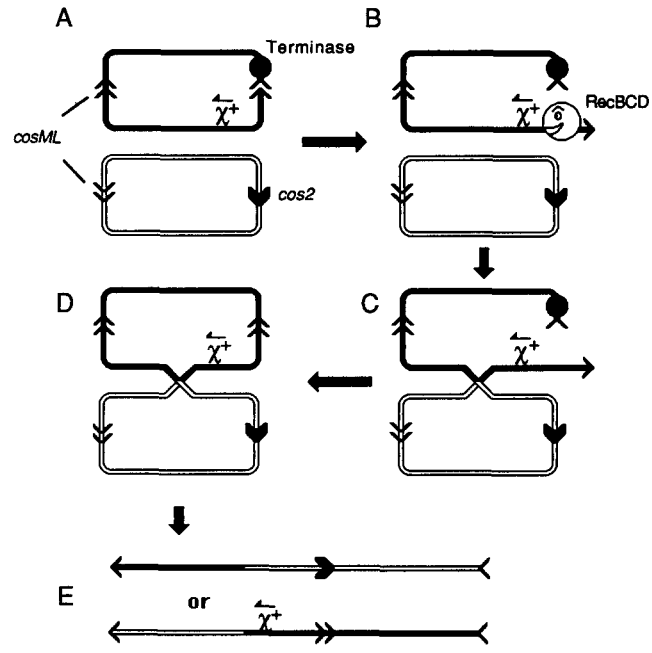

FIGURE 2.—Approximately equal recovery of complementary recombinants when one parent is *cos2* (KOBAYASHI *et al.* 1984). When the χ^0 parent has mutant *cos*, packaging is uncoupled from χ activation. A–C, as in Figure 1. The packaging initiated in C is unable to be completed because *cos* in the responding phage is an uncuttable mutant version (*cos2*). Consequently, packaging may reverse, and the *cos* that was opened to initiate recombination recloses (D). Packaging can then reinitiate on the resulting circular dimer. Depending on which cloned *cos* initiates the packaging, the χ^+ or the χ^0 recombinant is packaged (E).

allow for packaging of recombinants but cannot themselves activate χ because of their improper orientation relative to it (KOBAYASHI *et al.* 1982). χ was seen to be active in these crosses (KOBAYASHI *et al.* 1983), and the complementary recombinants were recovered more-or-less equally. These results argued that χ-stimulated recombination is reciprocal. They were interpreted as follows: The act of packaging that allowed for the entry of RecBCD enzyme is aborted because of *cos2* at the standard locus in the χ^0 parent. The DNA dissociates from terminase and the sticky ends reclose, creating an intracellular circular dimer. Subsequent packaging from the dimer, which goes (demonstrably) via the cloned *cos* sites, is unprejudiced, resulting in equal, though low, recovery of the complementary recombinants.

Several enzymatic activities of the RecBCD enzyme have been demonstrated *in vitro* [see TAYLOR (1988) for review]. Among them is an ATP-dependent exonuclease (ExoV) activity that degrades both chains of linearized double chained DNA. Models of RecBCD-mediated recombination have often ignored that fact because reciprocal recombination and double chain degradation are not easily reconcilable.

Under prompting from SHUMO LIU and A. V. KUZMINOV, we have reinvestigated the reciprocality of RecBCD-mediated recombination in λ crosses. Those two correspondents independently proposed that the linearization of λ consequent to *cos* cutting was likely to result in degradation of DNA by the exonuclease activity of RecBCD enzyme. The proposed degradation might be a side reaction of ExoV or might be due to the action of the very RecBCD enzyme molecule whose subsequent interaction with χ leads to recombination. With an eye on the latter possibility, they and we elaborated a model of THALER et al. (1988, 1989) by proposing that the RecBCD enzyme travels through DNA from its entry point at *cos* acting as both a helicase and a single-chain endonuclease, the combined activities that arguably constitute ExoV activity [for review, see TAYLOR (1988)]. Upon encountering a correctly oriented χ, or χ-like sequence, ExoV activity is suppressed by separation of the RecD subunit from the RecBCD enzyme heterotrimer (and see CHAUDHURY and SMITH 1984). The χ-modified enzyme [RecBC(D^-)], devoid of the RecD subunit and of ExoV activity, can move through DNA, with no net unwinding, and cut it in a manner that effects recombination. This model explains both the ability

of χ to stimulate recombination and the hyper-Rec activity of null mutants of *recD* (AMUNDSEN *et al.* 1986; BIEK and COHEN 1986; THALER *et al.* 1989). It derives from the remarkable set of observations, ascribable to GERRY SMITH'S laboratory, that *recD* mutants are hyper-rec while crude extracts from them lack ExoV and helicase activities. It receives further support from the conclusion (PALAS and KUSHNER 1990) that the RecBC(D^-) enzyme retains ATP-dependent endonuclease activity and some ability to render DNA sensitive to the single-chain exonuclease ExoI, at least in the presence of single-strand binding protein.

As remarked above, *cos*-cutting followed by ExoV-mediated DNA degradation would appear to preclude the recovery of equal numbers of complementary recombinants reported by KOBAYASHI *et al.* (1984). S. LIU and A. V. KUZMINOV (personal communications), however, independently suggested that the end of DNA created by ExoV digestion is highly reactive in recombination and usually engages a third phage chromosome. When that third chromosome is of the same parental genotype as the χ^+ phage that initiated the observed event, recombinants of complementary genotype will be generated (Figure 3). The complementary recombinants will be packaged from the resulting trimer. In this view, the equality of complementary recombinants reported by KOBAYASHI *et al.* (1984) requires the participation of two χ^+ parent chromosomes rather than one such chromosome as KOBAYASHI *et al.* proposed. LIU recognized that this feature of the model predicts that the equality in recovery of complementary recombinant types will obtain only when the χ^+ parent is present in excess over the χ^0 one. He gathered preliminary data supporting his view (S. LIU, personal communication).

The work reported here expands on LIU's experiment and fully confirms his preliminary results. Additional experiments support the conclusion that χ stimulates triparental events in the manner predicted by the model of LIU and KUZMINOV–we find that a λ chromosome that has incorporated a plasmid tends to undergo a λ × λ exchange in the interval in which the plasmid was incorporated.

MATERIALS AND METHODS

Phage genetic elements are described in Table 1, and their locations are shown in Figure 4.

Bacterial strains and plasmids are described in Table 2. *malB*::Tn*9* obtained from DON ENNIS was used to move the *dnaBts22* allele via P1-mediated transduction. All plasmids were present in the cultures employed in predominantly (>90%) monomeric state as judged from gel electrophoresis of cleared lysates followed by EtBr staining.

Density labels, replication blocks and gradient centrifugation are as in previous papers (*e.g.*, STAHL and STAHL 1986). Unless otherwise specified, crosses were conducted by combining 1.5×10^8 cells with 2×10^9 plaque-forming units of phage and aerating the mixture at an appropriate temperature. After 90 min, cell lysis was induced with chloroform plus lysozyme. When cross lysates were analyzed by gradient centrifugation, the entire 2-ml volume of the cross was combined with cesium formate.

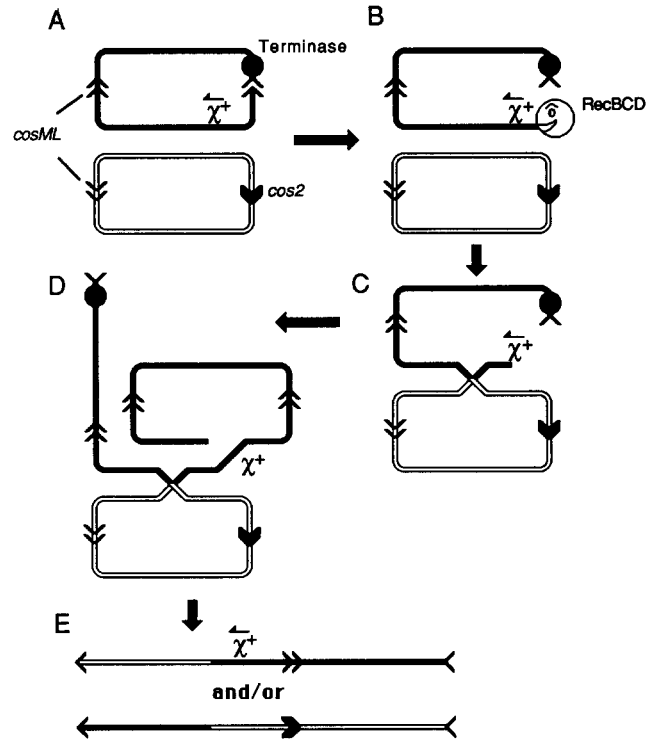

FIGURE 3.—Recovery of complementary recombinants via a triparental event (proposals of A. KUZMINOV and S. LIU, personal communications). A, as in Figures 1 and 2. B, RecBCD enzyme enters at the right end of the linearized λ, digesting the DNA. C, χ-stimulated recombination occurs left of the χ sequence. D, The degraded duplex end invades a third phage. When the third phage is a χ^+ parent type, complementary recombinant chromosomes will be formed in nearly equal amounts. (E) Since packaging from standard *cos* is blocked by the *cos2* mutation in the χ^0 phage, the recombinant chromosomes can be packaged only from the cloned *cos*'s.

Anomalies in density gradients: (1) Phage chromosomes carrying a large deletion or several smaller ones can be packaged in heads lacking the λD protein (STERNBERG and WEISBERG 1977). Such particles are viable and have a nonhereditary density that is greater than expected for particles carrying the deleted chromosome. Several of our gradients reveal a peak or shoulder of such anomalous particles on the heavy side of a peak of phage deleted for about 15% or more of the λ chromosome. This anomaly does not interfere with our analyses. (2) Our plasmid (pLT16) that has homology with λ*J* is deleted internally for part of *J*. We used this plasmid because its presence in λ minimally interfered with the scoring of clear vs. turbid plaques. In some experiments (*e.g.*, the ones in which one parent contains the plasmid) a minor shoulder on the heavy peak probably represents particles that have retained the plasmid but lost the deletion. (3) A third anomaly was avoided by growing stocks of phage with large deletions on the *recA* host FS2017. When the $recA^+$ ancestor (FS1576) of this strain was used with such phage, mutants of increased density, probably resulting from recombination with lambdoid DNA in the host chromosome, contaminated our stocks.

TABLE 1

λ Genetic elements employed

Element	Relevant properties	Reference/source
cos2	Deletion of cut site of *cos*	KOBAYASHI *et al.* (1982)
cos^{21}	hy33: *cosB*, *Nu1* equivalent, and part of *A* equivalent of phage 21	FRACKMAN, SIEGELE and FEISS (1984)
Asus11	Amber marker near λ's left end	CAMPBELL (1961)
Bts1	Heat-sensitive marker near λ's left end	BROWN and ARBER (1964)
Jsus6	Amber marker in *J*, left of *att*	CAMPBELL (1961)
cosML	Cloned *cos*, oriented backward	KOBAYASHI *et al.* (1982)
int4	Unconditional Int^- mutation	GINGERY and ECHOLS (1967)
red3	Unconditional Red^- mutation	SIGNER and WEIL (1968)
gam210 = γsus	Amber (*sus*) mutation in *gam* (γ)	ZISSLER, SIGNER and SCHAEFER (1971)
ΔC	Int^- Red^- deletion	CAMERON *et al.* (1975)
b1453	Deletion from *att* rightward into *gam*	HENDERSON and WEIL (1975)
b1319	Deletion from *att* rightward into *c*III	HENDERSON and WEIL (1975)
imm^{434}	Immunity region from phage 434	KAISER and JACOB (1957)
imm^{21}	Immunity region from phage 21	CAMPBELL (1971)
χ^+C	χ site in *c*II	STAHL, CRASEMANN and STAHL (1975)
*c*II*68*	Unconditional *c*II mutation	IRA HERSKOWITZ
Psus80	Amber mutation in *P*	CAMPBELL (1961)
nin5	Deletion	FIANDT *et al.* (1971)
χ^+D	χ site between *nin5* and *R*	STAHL, CRASEMANN and STAHL (1975)
Rsus5	Amber mutation in *R*	CAMPBELL (1961)
Rts129	Heat-sensitive mutation in *R*	CAMPBELL and DEL CAMPILLO-CAMBELL (1963)

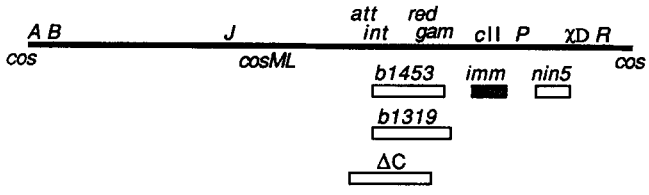

FIGURE 4.—Map of λ showing the features involved. Coordinates of many of these elements can be found in DANIELS *et al.* (1983).

RESULTS

Unequal input crosses with χ^+ in one parent: In the hypothesis that provoked these experiments, the χ-stimulated formation of the recombinant inheriting χ^+ requires the participation of two χ^+ parental chromosomes, while its complement, not inheriting χ^+, requires the participation of only one χ^+ chromosome (Figure 3). This feature of the model was tested by crosses in which the relative multiplicities of the two infecting parents were varied. The model predicts that complementary recombinants will be recovered approximately equally only when the χ^+ parent is present in excess. Otherwise, the χ^0 recombinant will be recovered preferentially.

The prediction that the relative production of complementary recombinants depends in a predictable way on the ratio of parental phage types is valid only if each of the chromosomes participating in a given recombination event is chosen at random with respect to its genotype. When chromosomes are free to replicate, this assumption is invalid because of "finite input" (LENNOX, LEVINTHAL and SMITH 1953) and is further endangered by a possible lack of panmixis (HERSHEY 1958). For λ recombining by the RecBCD pathway, these nonidealities may be responsible for the failure to see 50% recombinants between terminal markers when dimerization by recombination appears to be required for packaging (STAHL 1979, pp. 297–298). Since the assumption of randomness in selection of partners is critical to a kinetic analysis, our crosses were conducted with DNA replication genetically blocked. The parental phages were density-labeled and the progenies were centrifuged in cesium formate equilibrium density gradients to confirm the adequacy of the replication block. Recombinant yields were determined by summing the titers in these density fractions.

Prior to undertaking the kinetic analysis, we verified the properties of the system, diagramed in Figure 5. On a single host cell culture, we executed four crosses, each at two different relative multiplicities of infection (10:5 and 0.5:7, respectively) (Figure 6). (1) Both parents were χ^0 and both *cos* sites in each phage were functional. The results (Figure 6, B1 and B3) demonstrate equal recovery of complementary recombinants at each moi. This result establishes that the complementary recombinant genotypes (ΔC imm^{21} and *b1453* imm^{434}) are equally viable. (2) The ΔC imm^{434} parent carries χ^+C, just to the right of imm^{434} and overlapped by imm^{21}. The results (Figure 6, B2 and B4) reveal an excess of the χ^0 recombinant (ΔC imm^{21}) over its χ^+ complement. The excess is greater when the χ^0 parent predominates in the infecting phage mixture. (3) The ΔC imm^{434} parent caries χ^+C while the χ^0 parent is mutant at standard *cos*. KOBA-

TABLE 2

Bacterial strains and plasmids

Strain No.	Relevant properties	Source/reference
594	Su⁻ *rec*⁺	WEIGLE (1966)
AC417	594 *recD1014*	A. CHAUDHURY
AFT196	Su⁺ *recA56*	A. F. TAYLOR
FA77	Su⁻ *dnaBts22*	MCMILIN and RUSSO (1972)
V66	Su⁻ *recF143*	CHAUDHURY and SMITH (1984)
V227	SuII *recC1001* (P2); selectively plates Spi⁻λ, giving large plaques	G. R. SMITH
FS1189	λ imm^{434} lysogen of SuII *recA* strain QR48	
FS1430	λ imm^{434} lysogen of *nusA1* strain	
FS1576	SuII *recD1009*	
FS1607	594[pKC31, monomer]	
FS1827	FA77 *lexA3*	
FS1980	V66 *dnaBts22*	
FS2017	SuII *recD1009ΔrecA306*::Tn*10*	
FS2520	FA77 *recJ284*::Tn*10*	
FS2720	V227(λimm^{21})	
FS2765	594[pBR325]	
FS2766	FA77[pBR325]	
FS2854	FS1980[pLT16, monomer]	
FS2942	FS2854 *dnaB*⁺	
pKC31	λdv carrying Kan^R (6.1 kb)	R. N. RAO
pLT16	Deletion derivative of pRK101 homologous to λ*J* and rightward (7.2 kb)	pRK101 is from M. S. FOX

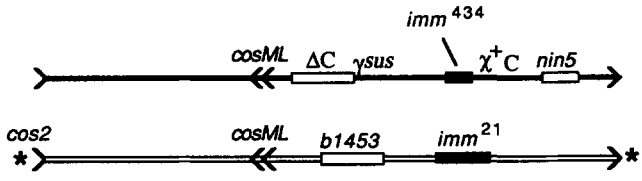

FIGURE 5.—λχ⁺ × λχ⁰ cross to measure the relative frequencies of complementary recombinant types arising in the RecBCD pathway. The complementary recombinant types Δ*Cγsus* imm^{21} and *b1453* imm^{434} can be separately enumerated. χ activity can be assessed by comparing the frequency of those recombinants with the frequency of the recombinant imm^{21} *nin5*, which is not stimulated by χ⁺C. The *cos2* mutation (*) in the χ⁰ parent dissociates the activation of χ⁺C from packaging of the resulting recombinants. The *cos2* mutant parent is here shown with the same gene order as the *cos*⁺ parent. In fact, since *cos2* is deleted for the terminase cut site, all *cos2* mutant phage particles carry chromosomes that are opened at *cosML*.

YASHI *et al.* (1983) showed that the introduction of *cos2* into a χ⁰ parent reduces the inequality of complementary recombinant types while retaining χ activity. As did KOBAYASHI *et al.*, we see (Figure 6, A2 and A4) a reduction in the nonequality of complementary recombinants. The two types are nearly equal when the moi is 10 χ⁺ parent to 5 χ⁰ parent (Figure 6, A2). Considerable inequality in the yields of complementary recombinant types remains, however, when the χ⁰ parent is in excess (Figure 6A4). This result confirms S. LIU'S (personal communication) observation and demonstrates the feasibility of the kinetic analysis with these phage types. (4) The corresponding χ⁰ crosses (Figure 6, A1 and A3) show only small inequalities in recombinant types. The reduction in yields upon addition of *cos2* to the χ⁰ parent in the χ⁰ × χ⁺ crosses (A2 *vs.* B2 and A4 *vs.* B4) indicates that about 10% of the recombinants initiated by cutting of standard *cos* succeed in packaging from cloned *cos*, as seen by KOBAYASHI *et al.* (1983). This implies that most of the recombinants in the χ⁰ crosses of A1 and A3 are initiated from terminase cutting of cloned *cos*. Since the two phages are equivalent with respect to χ (both are χ⁰) and cloned *cos*, the near equality of recombinant types is expected from the previously demonstrated equivalence in the viability of the complementary recombinant genotypes.

The results of Figure 6 established the credentials of the system, and the kinetic analysis was undertaken.

In the kinetic analysis, multiplicities of the χ⁺:χ⁰ *cos2* parent were varied from 12:1 to 1:12, while total moi was held constant at 13. Crosses were density labeled and replication-blocked, allowing us to verify that the block was maintained at all relative moi's. In Figure 7, the fractions of the recombinant types that were χ⁺ are plotted *vs.* the fractions of input parents that were χ⁺. The line is the expectation based on the following simple assumptions: (4) All events in the marked interval were initiated by the χ⁺ parent, (2) two χ⁺ parents were required for formation for the χ⁺ recombinant while one was required for formation of the χ⁰ recombinant, and (3) the two recombinants were equally likely to be packaged. We suspect that the deviation of the data from the line at low moi of the χ⁺ parent results from the fact that a (minor) fraction of the recombination events in the marked interval initiated from the cloned, inverted *cos*. Such recombinants are equally likely to be χ⁰ or χ⁺ since

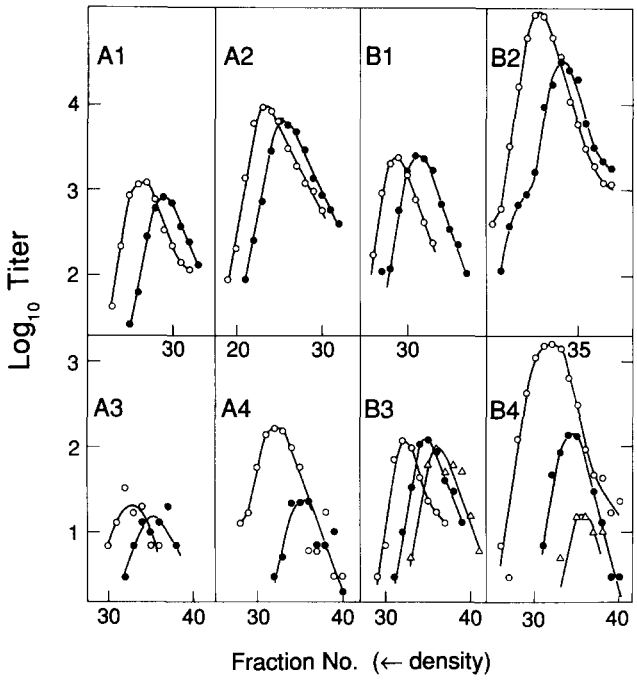

FIGURE 6.—Effects of relative multiplicity of infection (moi), *cos2*, and χ^+C on the recovery of complementary recombinants. Crosses like those in Figure 5 were conducted with blocked DNA replication in the Su$^-$ *dnaBts* host FA77 at 40°. Phages were density-labeled (heavy × heavy) to verify the replication block. Fractions from cesium formate equilibrium gradients were surveyed for phage titer by spot tests. Two peaks, corresponding to unadsorbed phage and to progeny carrying fully conserved chromosomes, respectively, were seen in all crosses. The progeny peak from each cross was plated for recombinant types. The complementary recombinants differ slightly from each other in density because of the different sizes of the deletions and substitutions that they carry. A, *imm*^{21} parent is *cos2*: 1, both parents χ^0; moi of *imm*^{434} parent = 10, moi of *imm*^{21} parent = 5. 2, *imm*^{434} parent χ^+; moi as in 1. 3, both parents χ^0; moi of *imm*^{434} parent = 0.5, moi of *imm*^{21} parent = 7. 4, *imm*^{434} parent χ^+; moi as in 3. B, both parents *cos*$^+$; 1–4, as in A. *Key*: ○, ΔC *γsus imm*^{21} on FS1189; ●, b1453 *imm*^{434} on FS2720; △, *imm*^{21} *nin5* on FS1430 at 40°.

TABLE 3

Recovery of complementary recombinants when DNA replication is permitted

χ	moi ΔC *γsus imm*^{434} $\chi^{+/0}$ / moi Δb1453 *imm*^{21} *cos2*	yield Δb1453 *imm*^{434} $\chi^{+/0}$ / yield ΔC *γsus imm*^{21} Expt. I	Expt. II	Avg.
χ^0	12/2	0.99 (380/382)	0.91 (210/231)	0.95
	2/12	0.95 (566/595)	1.30 (524/402)	1.13
χ^+	12/2	0.91 (161/177)	1.19 (192/161)	1.05
	2/12	1.19 (407/343)	1.06 (510/483)	1.13

The phage depicted in Figure 5 were crossed in the Su$^-$ *rec*$^+$ strain 594. Complementary recombinants were scored in FS2720 and on FS1189, respectively. Numbers in parentheses are plaques counted in determining the recorded ratios.

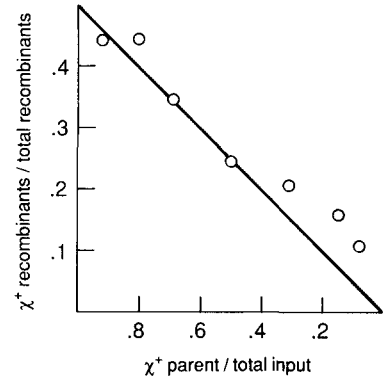

FIGURE 7.—Relative recovery of complementary recombinants as a function of relative moi of parents. Conditions as described in Figure 6 except *imm*^{434} parent was always χ^+, *imm*^{21} parent was always *cos2*. Total moi was held constant at 13 while relative moi of parents varied. Titers of recombinants were calculated from peaks like those in Figure 6 (A2 and A4).

χ^+C cannot be activated by an inverted *cos*.

When DNA replication was permitted, complementary recombinants were recovered equally without regard to the relative moi's of the infecting parents (Table 3). This apparent reciprocality may imply an artifactual basis to our replication-blocked crosses. On the other hand, it may reflect merely the anticipated difficulty in controlling the ratios of parental types in individual cells (LENNOX, LEVINTHAL and SMITH 1953) (or regions of cells, HERSHEY 1958) when replication is allowed. In some schemes, these two possibilities may not be distinguishable, even in principle. For instance, *cos* may be most easily cut by terminase on a chromosome that has just replicated. Such a rule would ensure the proximity of a sister χ^+ chromosome to the χ^+ chromosome responsible for the observed recombination. It is likely that part of the loss of inequality in complementary recombinant types is a result of the reduction in χ activity (relative to background) seen in replication allowed crosses (STAHL, KOBAYASHI and STAHL 1983; Figure 12, this paper). Exchanges occurring independently of χ will yield complementary recombinants equally.

The model that motivated these experiments (Figure 3) features a (probably frayed) double chain end, resulting from ExoV-catalyzed DNA degradation, invading an intact duplex. The RecF pathway has been viewed as operating on double chain breaks (*e.g.*, see THALER *et al.* 1989), which raises the question of whether RecF pathway genes might be involved in the formation of the χ^+ recombinant in these crosses. Results of crosses with varying multiplicities performed in *recF* and in *recJ* mutant cells (FS1980, FS2520, respectively) were like those obtained with rec$^+$ cells. They gave equal numbers of complementary recombinants, but only when the χ^+ parent was in excess (Table 4). Thus, there is no evidence of RecF pathway involvement in this phenomenon. Previous

TABLE 4

Effect of relative moi on fraction of complementary recombinants that are χ^+ in *recF* and *recJ* mutant cells

moi ΔC *γsus imm*^{434} χ^+ / moi Δb*1453 imm*^{21} *cos2*	yield Δb*1453 imm*^{434} χ^+ / yield ΔC *γsus imm*^{21}		
	FA77 (*rec*$^+$)	FS1980 (*recF*)	FS2520 (*recJ*)
11/2	0.45	0.49	0.48
2/11	0.16	0.20	0.25

The cross of Figure 5 was conducted in the indicated *dnaBts rec* mutant host cells and analyzed as in Figure 7. The data for FA77 are from Figure 7.

TABLE 5

Effect of relative moi on fraction of complementary recombinants that are χ^+ in *lexA3* mutant

moi ΔC *γsus imm*^{434} χ^+ / moi Δb*1453 imm*^{21} *cos2*	yield Δb*1453 imm*^{434} χ^+ / yield ΔC *γsus imm*^{21}	
	FA77 (*rec*$^+$)	FS1827 (*lexA3*)
12/2	0.44	0.23
2/12	0.23	0.03

The cross of Figure 5 was conducted in the indicated *dnaBts* hosts and analyzed as in Figure 7. The four crosses were performed in parallel.

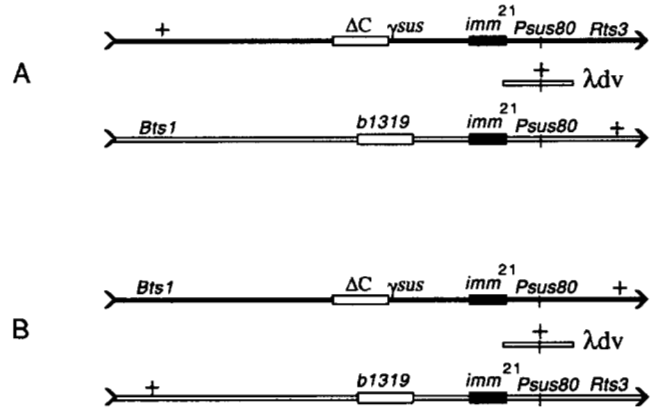

FIGURE 8.—λ × λdv × λ crosses. Progeny phage were sorted by density, which separates particles that have or have not spliced the plasmid into λ. In the light fraction, selection for P^+ detected phage that have "patched" in P^+ information. Plating at high temperature selected B^+R^+ recombinants. These recombinants are scored for ΔC *γsus vs. b1319* to determine whether the exchange that generated the Ts^+ recombinant occurred in the left interval or the right one, which contains the *P* gene. The relative frequencies of these two recombinant types can be compared for phage that have or have not recombined with the plasmid.

work has shown that *recF* mutations have no effect on "RecF" pathway recombination in lytic cycle λ crosses but that *recJ* mutations depress such recombination (STAHL *et al.* 1986; THALER *et al.* 1989).

SOS functions are induced in *dnaBts* cells at high temperature (SCHUSTER *et al.* 1973; S. LIU and D. S. THALER, personal communication). Such induction is not responsible for the observed dependency on relative moi, since we find a dependency with host cells (FS1827) that are noninducible by virtue of the *lexA3* mutation (Table 5). The *lexA3* cross, however, gives a larger inequality of complementary types at both moi's, suggesting that an overproduction of RecA protein (or some other SOS-inducible protein) secures the secondary exchange envisaged by the model. The yield of the χ^0 recombinant in the *lexA3* mutant was 2.0 × less than that from the *rec*$^+$ cross when the moi was 2:12 and 7.1 × less when the moi was 12:2, suggesting that the primary recombination event is similarly affected by the *lexA3*.

Recombination of λ with plasmid: Our λ × plasmid crosses have the following general features: They are conducted in cells carrying a monomeric circular plasmid that has homology to λ. By virtue of deletions, the λ chromosomes have room to pick up one (only) copy of the plasmid while remaining packageable. Markers define a control interval and an interval in which the plasmid can be picked up. Particles carrying chromosomes that have picked up a plasmid are detected by the resulting increase in particle density. We compare the relative λ × λ recombinant frequencies in two intervals, the one in which pickup occurs and a control interval, among recombinant phage that have or have not recombined with the plasmid, respectively. The markers defining the two intervals are switched to guard against marker effects.

The model (Figure 3) features two close exchanges, a reciprocal one to the left (3C) and a nonreciprocal one to the right (Figure 3D). In a λ × plasmid × λ cross, the reciprocal event will pick up a plasmid while the nearby nonreciprocal event will occur as a result of the same act of *cos*-cutting and will render packageable the chromosome that picked up the plasmid.

λ*dv plasmid:* The crosses are diagrammed in Figure 8. The model (Figure 9) predicts that recombination of λ with the λdv plasmid results in a λ chromosome that is degraded at its right end and is highly reactive for recombination with another λ chromosome. When the initial pickup has been into the *Bts* parent, the λ × λ event that follows the pickup will not generate a P^+ Ts^+ recombinant, since the resulting phage will almost certainly be *Bts*. When the pickup has been into the *Rts* parent, however, the correlated λ × λ event will frequently result in a P^+ Ts^+ recombinant by virtue of exchange occurring to the right of the pickup. Therefore, the model predicts that Ts^+ recombinants that have become P^+ by picking up either the plasmid or the P^+ marker therefrom will be more highly recombinant in the interval containing the *P* gene than will other Ts^+ recombinants. Data (Figure 10) support that expectation. Among P^+ λ, whether in the heavy, plasmid pickup peak or in the standard

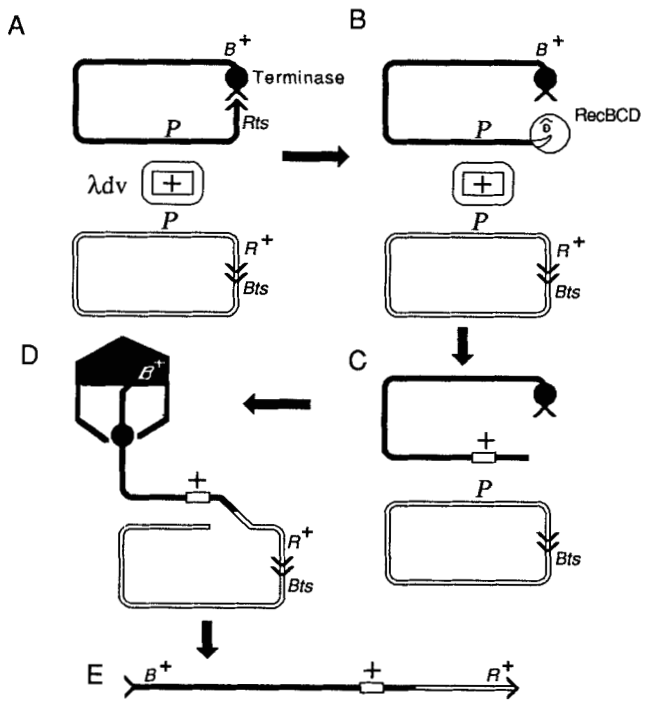

FIGURE 9.—Expectation for the crosses of Figure 8 according to the model of A. KUZMINOV and S. LIU. In a cross of λ × λdv × λ (A), one λ chromosome is opened at *cos* allowing entry of the RecBCD enzyme with consequent digestion of DNA (B). If the RecBCD enzyme loses the RecD subunit before the protein passes the *P* region, it can catalyze recombination with λdv leading either to incorporation of the plasmid or to the pick up of P^+ without plasmid incorporation (C). The DNA end remaining from digestion by ExoV (C), attacks a λ chromosome (D) leading to a packaged B^+R^+ recombinant that has either spliced or patched with the plasmid (E).

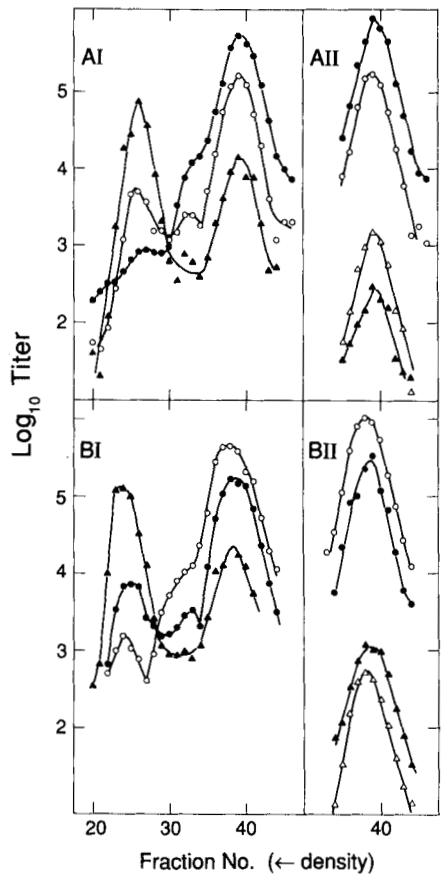

FIGURE 10.—Crosses A and B of Figure 8 were performed in the Su^- strain FS1607 with seven infecting phage of each type per cell. Lysates were centrifuged in cesium formate and plated as follows. Panels AI and BI: ●, at 42° on the Su^+ *recC1001* P2 lysogen V227 for Ts^+ recombinants that inherit *b1319*; ○, at 42° on the Su^+ *recA* host AFT196 for Ts^+ recombinants that inherited ΔC *γsus*; and ▲, at 32° on the Su^-*recD* strain AC417 for total P^+. Panels AII and BII, in the region of the light peak only. At 42° on the Su^+ *recD* host FS1576: ●, clear plaques (Ts^+ phage that inherit *b1319*) and ○, turbid plaques (Ts^+ phage that inherit ΔC *γsus*). At 42° on the Su^-*recD* host AC417: ▲, clear plaques (P^+Ts^+ phage that inherit *b1319*) and △, turbid plaques (P^+Ts^+ phage that inherit ΔC *γsus*). The heavy P^+ peak is of λ that have incorporated the plasmid, while the light P^+ peak is of λ that have "patched" P^+ information from the plasmid. In panels A1 and B1, the interval in which the Ts^+-generating crossover occurred was scored by plating that was selective for *b1319* (Spi^-) or ΔC Gam^+ (Fec^+), respectively. (The presence of λdv in the phage chromosome interfered with the clear vs. turbid phenotypes of *b1319* and ΔC*γsus*, respectively.) In panels A2 and B2, *b1319* and ΔC*γsus* are scored as clear *vs.* turbid plaques, respectively. All phages are imm^{21} to allow for growth in the presence of λdv. The deletions serve not only as markers defining the two intervals to be compared but also provide space for the plasmid (which is predominantly monomeric in this strain) to be picked up. In the presence of these deletions some viable phage particles are produced that lack λ D protein, resulting in heavy shoulders (or peaks) to the left of the major light peaks in AI and BI.

density peak, the fraction of Ts^+ recombinants that have crossed over in the *b1319-R* interval is higher than in the overall Ts^+ λ population. The change in the distributions of exchanges due to picking up the plasmid can be calculated from the peak heights of Figure 10 (A1 and B1). In the overall Ts^+ population, 25% of the exchanges occurred in the *b1319* to *R* interval (average of A and B). Among phage that have picked up the entire plasmid, the fraction of Ts^+ recombinants exchanged in that interval is 84%. The change in exchange distribution due to picking up P^+ information without picking up the plasmid can be calculated from Figure 10 (A2 and B2). In the overall Ts^+ population, 19% of the exchanges occurred in the *b1319-R* interval (judged from peak heights). Among phage that have picked up P^+ from the λdv, the fraction of Ts^+ recombinants exchanged in that interval is 76%. The differences between 25% and 19% on the one hand and between 84% and 76% on the other may be partly technical–as described in Figure 10, different plating bacteria were used to collect the two sets of data.

Our results show that λ which have recombined with λdv have an atypical distribution of crossovers in their λ × λ exchanges, ruling out the possibility that recombination of λ and λdv occurs independently of the recombination of λ × λ. The distribution observed is that expected from the model. Equivalent experi-

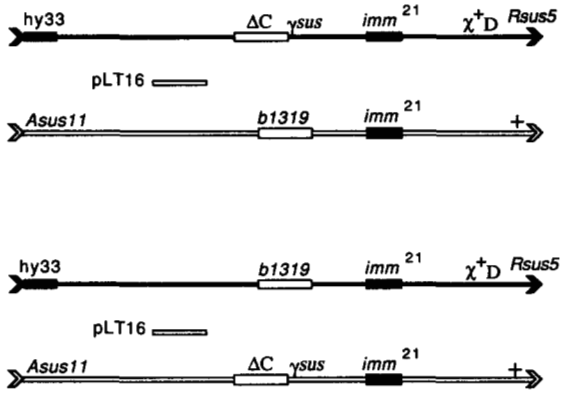

FIGURE 11.—λ + PLT16 × λ crosses. All recombination events initiate on the cos^{21} parent. Progeny phage are sorted by density to identify those that have picked up the plasmid. cos^{21} R^+ recombinants are scored for ΔC *γsus vs. b1319* in the plasmid pick-up peak and in the control peak.

ments using a colE1-based plasmid carrying λ's P^+ gene gave results like those obtained with λdv (data not shown).

Plasmid near J: A second test of the model is afforded by a cross in which the plasmid to be picked up must recombine with sequences located in the left member of two marked intervals. The model supposes that plasmid pick up is the result of RecBCD enzyme entry into a linearized λ followed by DNA degradation to variable extents leftward before the protein loses exonuclease activity and becomes competent to commit to recombination homologously paired regions of DNA. The frayed end created by this degradation will initiate an exchange with another λ chromosome, sometimes resulting in the selected Sus^+ recombinant. Thus, among Sus^+ recombinants, the λ × λ exchange occurring to the right of the incorporated plasmid will tend to occur more often in the left interval among recombinants manifesting plasmid pick-up than among those that have not recombined with the plasmid.

Our crosses are diagrammed in Figure 11. They have the following additional features: (1) One parent is mutant in a terminase gene (*Asus11*). The other parent carries the heterospecific substitution cos^{21} (hy33), which is allelic to *Asus11*. Since the crosses are conducted in a Su^- host, only a minor fraction of the terminase-dependent recombination and packaging can initiate on the *Asus11* parent (FRACKMAN, SIEGELE and FEISS 1984; I. SIDDIQI, F. W. STAHL and M. M. STAHL, in preparation). (2) The cos^{21} parent is *Rsus5*, so that Sus^+ λ × λ recombinants can be selected among the progeny. (3) The interval that has homology with the plasmid has a low recombination rate relative to that of the control interval, to the right, which contains χ+D. This relatively low rate facilitates the detection of a rate increase accompanying pickup of the plasmid. Within the framework of the model elaborated in the Introduction (see Figure 3), we anticipate that the plasmid will typically be picked up independently of χ+D, which is far (about 25 kb) to the right of the region of λ that is homologous with a segment of the plasmid. (In the APPENDIX we show that when λ carries two χ both are active, although the χ on the left has reduced activity. Thus, not all recombination to the left of χ+D in the initiating parent is dependent on χ+D.) (4) The λ × λ event required to make the selected Sus^+ recombinants will tend to occur in the interval with the pickup if the responsible RecBCD enzyme molecule degraded the λ chromosome from the right end into the interval in which pickup occurs.

Among phage that have not picked up the plasmid, our data for replication-blocked crosses (Figure 12, A1 and B1) show that about 3% of the Sus^+ recombinants are the result of exchange in the *A*-ΔC interval. Among those that have picked up the plasmid in that interval, about 24% of the Sus^+ recombinants have recombined there. Within the framework of the model, the increased λ × λ recombination is due to exchanges in the much shorter, plasmid-ΔC interval. The selected recombinants in these λ × plasmid × λ crosses, although trimolecular, are presumed by the model to be bimolecular in λ. Any nonidealities resulting from "finite input" should affect the control and experimental intervals equally. We anticipate, therefore, that the results of these experiments will be independent of whether or not DNA replication is blocked and independent of the relative moi of the two parents. These expectations were realized (Figure 12). (In the replication allowed crosses (Figure 12, A2 and B2), the fraction of Ts^+ recombinants resulting from exchange in the right hand interval is reduced relative to that observed for replication-blocked crosses. This reduction of χ activity by DNA replication has been reported (STAHL, KOBAYASHI and STAHL 1983), and probably reflects the existence of replication-dependent entry points for RecBCD enzyme.)

Phage that have previously incorporated pLT16 do not have a high rate of λ × λ recombination in the *A*-ΔC interval or in the plasmid-ΔC interval (Figure 13). When the A^+ parent enters the cross already carrying plasmid, most of the B^+ R^+ recombinants retain the plasmid as shown by their density. Ninety-five percent of these plasmid-bearing B^+R^+ recombinants have crossed over in the *b1319-R* interval. These results demonstrate that it is the act of picking up the plasmid, not the presence of the plasmid in the λ chromosome, that gave the high rates of recombination in the *A*-ΔC interval in Figure 12. Among phage that have lost the plasmid there is a shift of exchanges into the *A*-ΔC interval. The mundane interpretation of this effect is that a principle route to plasmid loss is unequal crossing over between plasmid-bearing λ chromosomes.

DISCUSSION

Our experiments were conducted as tests of a proposal by SHUMO LIU and A. V. KUZMINOV. This

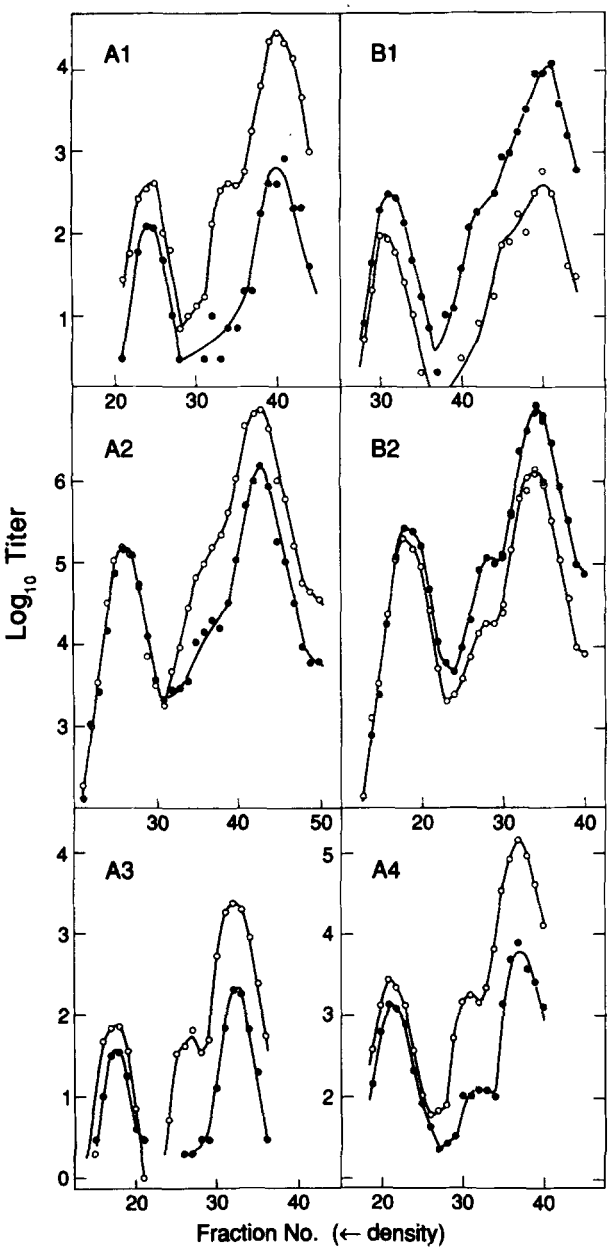

FIGURE 12.—Results of the crosses described in Figure 11. The progeny lysates were centrifuged in cesium formate to separate particles that had picked up the plasmid (heavy peak) from those that had not (light peak). The density fractions were plated on the Su⁻ strain AC417, and plaques were scored as clear (●) or turbid (○). In cross A, crossing over to the left of ΔC gives clear plaques while crossing over to the right of *b1319* gives turbid plaques. In cross B, the markers are reversed. The deletions serve not only as markers defining the two intervals to be compared but also provide space for the plasmid to be picked up. Heavy shoulders on the light peaks are as in Figure 10. A1 and B1, replication blocked crosses (moi = 7 for each parent) in the Su⁻ *dnaBts22* host FS2854. A2 and B2, free replication crosses (moi = 7 for each parent) in FS2942, a *dnaB*⁺ derivative of FS2854. A3 and A4, replication blocked crosses in FS2854 with unequal parental moi. A3 is 2 cos^{21} χ⁺D:10 *Asus*; A4 is 10 cos^{21} χ⁺D:2 *Asus*.

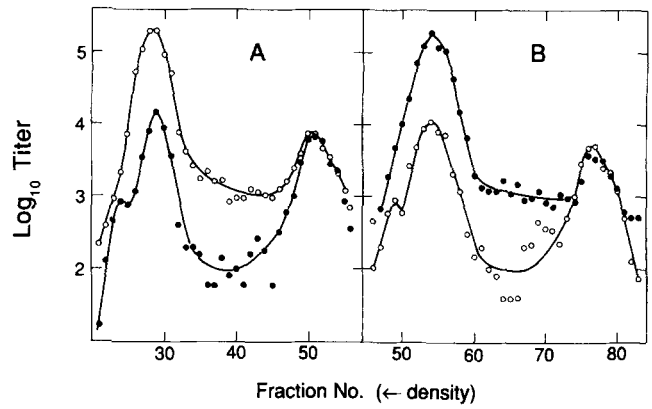

FIGURE 13.—Each of the cos^{21} parents from the crosses in Figure 11 was grown in FS2942. The lysates were centrifuged, single plaques were selected from the heavy peaks, and stocks were grown on the RecA⁻ host FS2017 to promote retention of the plasmid. In the phage stocks obtained, more than 90% of the particles carried the plasmid as verified by density gradient centrifugation. Each of these phage stocks was then crossed to the appropriate *Asus11* stocks not carrying plasmid, lysates were centrifuged, and *sus*⁺ progeny were scored as clear (●) or turbid (○) for the *b1319* or ΔC markers, respectively. A, cos^{21} ΔC γ*sus* parent with plasmid × *Asus11 b1319* parent. B, cos^{21} b1319 parent with plasmid × *Asus11* ΔC γ*sus* parent. In A and B the heavy shoulders on the peaks of plasmid-bearing phage that have recombined in the plasmid-bearing interval are probably a consequence of elimination, by unequal crossing over, of the deletion in the cloned λ fragment of pLT16.

model, a modification of that by THALER *et al.* (1989), proposes that ExoV is active until the traveling RecBCD enzyme loses its D subunit, which happens with heightened probability when the enzyme interacts with χ. The RecBC(D⁻) enzyme, devoid of its helicase and hence its ExoV activity, continues to travel (leftwards in λ) until it effects a reciprocal exchange somewhere to the left of χ. One of the two products of this exchange is incomplete, due to ExoV-catalyzed degradation from *cos* to χ. The recovery of viable χ⁺ recombinant phage particles is dependent on the (RecA-catalyzed) reactivity of the frayed end resulting from that degradation. Our experiments resulted in observations in agreement with predictions of the model.

Some of our experiments are compatible with a model proposing that the primary exchange itself, rather than being bimolecular and reciprocal, is trimolecular and is composed of a pair of individually nonreciprocal interactions (Figure 14). This model is compatible with the data from the λ × λ crosses with varying input as well as with the crosses involving pickup of λdv. For the plasmid pickup crosses (in the *A*-ΔC interval), however, this model predicts that all of the *hy33* R⁺ particles that have picked up a plasmid will be recombinant in the *A*-ΔC interval (see Figure 11), in contradiction to the observed 24%.

Our λ × λ crosses are probably interpretable within the framework of an *ad hoc* rule for chromosome packaging–perhaps all packaging, under our conditions, requires trimeric, instead of dimeric DNA as is usually supposed. Were that the rule, all progeny chromosomes in our replication blocked crosses would

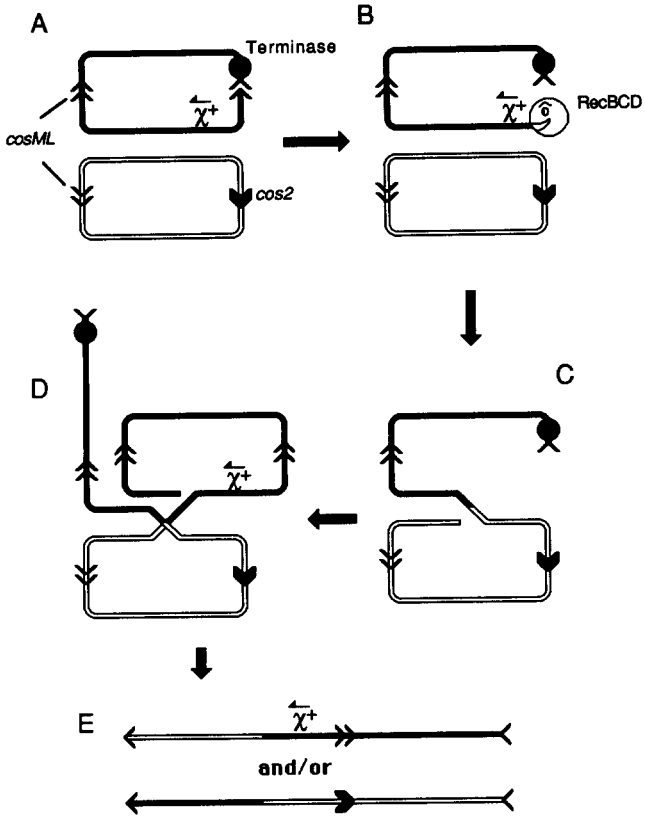

FIGURE 14.—A model in which the primary RecBCD-mediated exchange is triparental. A and B as in Figures 2 and 3. C, the χ-stimulated exchange is nonreciprocal. An unjoined end from the responding phage seeks a third chromosome and effects nonreciprocal recombination with it. When the third chromosome is χ^+ complementary χ-stimulated recombinants are recovered approximately equally.

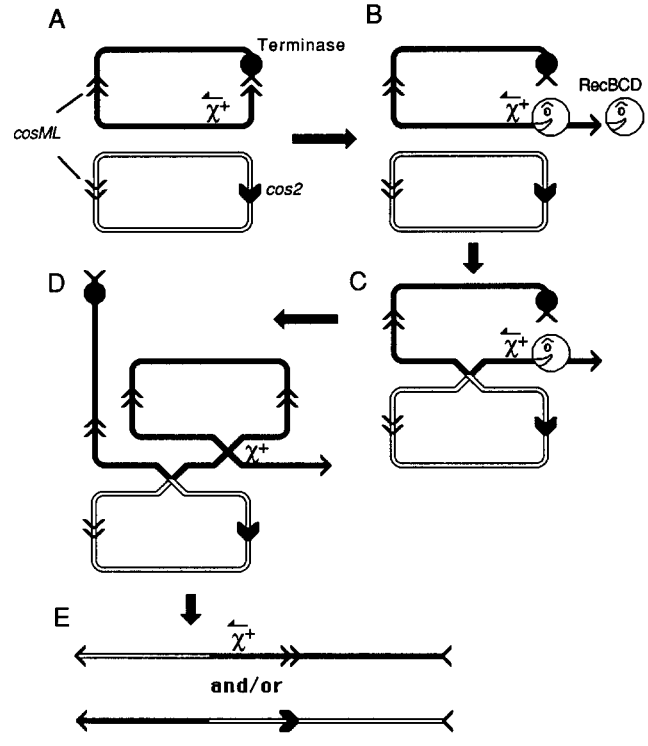

FIGURE 15.—The requirement for two χ^+ chromosomes in the formation of the χ^+-containing recombinant could reflect an imagined requirement for trimerization prior to packaging. In this scheme, the two exchanges needed for trimer formation are effected by two separate RecBCD enzyme molecules invading a χ^+ chromosome opened at *cos*.

perforce come from tri-parental interactions. The two crossovers that generated the trimer could be catalyzed by separate RecBCD enzyme molecules stimulated by the same χ in the originally linearized chromosome. The crossovers would generate complementary recombinants in the monitored interval when the χ^+ parent was in excess (Figure 15). This rule, however, doesn't appear to offer an explanation for the results of the λ × plasmid × λ crosses. Furthermore, we see no basis for the proposed inability to package at least one chromosome from a dimer.

Our present working hypothesis for RecBCD and χ differs from that offered by THALER *et al.* (1989) primarily by supposing that Exo V is fully active until the RecD subunit falls off and that the end created by ExoV activity is highly reactive. As in the hypothesis of THALER *et al.* (1989), the enzyme devoid of the D subunit continues to travel, without net unwinding of DNA, until it encounters either (1) a RecA-mediated paranemic 4-chained structure (CONLEY and WEST 1989) or (2) such a structure that has been converted to a double Holliday junction (KOBAYASHI and IKEDA 1983) by the action of a topoisomerase. In the first case, a proposed nucleolytic activity of the RecBC(D^-) enzyme cuts the paranemic structure, converting it to a single Holliday junction (HOLLIDAY 1964). Resolution of this intermediate may or may not involve the RecBC(D^-) enzyme. In the second case, RecBC(D^-) acts to cut at least one of the two junctions.

Under some conditions, the model (Figure 3) allows for nonequality in the recovery of complementary types even when the χ^+ parent is present in great excess. The χ^0 recombinant might be packaged directly from its own *cos* or, processively (*e.g.*, FEISS, SIPPY and MILLER 1985), in a linked sequence of packaging acts starting at the *cos* of the χ^+ recombinant. The χ^+ recombinant, on the other hand, can be packaged only from a packaging sequence that starts at its own *cos*. If both *cos*'s acted equally and if processivity were perfect, the model anticipates that the χ^+ recombinant could never be greater than one-third of all recombinants. Our data (Figure 7) do not reveal such a limitation, suggesting either that the model is flawed or that packaging is not processive under our conditions (RecBCD^+, replication blocked, temperature 42°, packaging from *cosML*).

The apparent reciprocality of χ-stimulated recombination (KOBAYASHI *et al.* 1984) led THALER and STAHL (1988) to propose a "*Tern*style" at *cos*, responsible for inhibiting ExoV activity when *cos* is cut.

However, if ExoV-catalyzed degradation of DNA does occur when *cos*-cutting initiates recBCD-mediated recombination of λ, as suggested by the present work, the *Ter*nstyle concept is superfluous.

MAHAN and ROTH (1989) observed paired splices involving three duplexes in RecBCD-mediated recombination in *Salmonella typhimurium*. Their interpretation of the events underlying the clustering is different from ours. Thus, there may be more than one cause of clustering, or the two demonstrations may find a common explanation. In either case, evidence for clustered splices offers the opportunity to reappraise some previous work.

Patches were defined by STAHL (1979, p. 82) as short, single chained segments of DNA donated from one duplex to its homologous position on another, creating a segment of duplex "hDNA" (which might subsequently be rectified by mismatch correction enzymes). For a marked locus, a patch may be detectable as information transferred from one chromosome to its homolog without disturbance of flanking markers. However, a recombinant that appears to be the consequence of a patch is not generally distinguishable from one that has arisen by a close pair of splices, which are the regions of hDNA arising from the joining of two duplex segments of parental DNA (STAHL 1979, p. 82). In practice, the distinction is made only for a population of recombinants and rests on the observation of localized negative interference (STAHL *et al.* 1982, STAHL, LIEB and STAHL 1984). Analysis of recombination in eukaryotes leads to the view that splices interfere with each other (for review, see STAHL 1979, pp. 111–112), and our knowledge of λ recombination mediated by λ's own pathway is compatible with that view (HERSHEY 1958). Patches, on the other hand, will contribute localized negative interference–*i.e.*, with a patch it is "unexpectedly" easy to contribute information from a chromosome to its homologue without perturbing the parental arrangement of flanking markers. With "patches" so defined, it was concluded that χ stimulates the giving and taking of "patches" equally (STAHL, LIEB and STAHL 1984).

The observation of STAHL, LIEB and STAHL (1984) was discussed as being compatible with the idea that". . .Chi may facilitate the resolution of a recombinational intermediate, such as a Holliday junction. Any nonreciprocality in a given junction (such as 'asymmetric hybrid DNA') would bear no relation to which parent carried χ." The work offered here emphasizes the possibility that the "patches" enumerated in the experiments of STAHL, LIEB and STAHL were mostly double splices, reducing the significance of the remarked compatibility. Reference to Figure 3 reveals a route by which χ could "give" a "patch" that was, in reality, a double splice. In Figure 3, if the third phage were white (χ^0), one of the packageable recombinants would be white with a "patch" of black.

ROSENBERG (1987, 1988) examined heteroduplexes that were parental for flanking markers in a challenge of the nick-at-χ model of SMITH *et al.* (1984). Paired splices, which could be scored as "patches," may confound such analyses. ROSENBERG's observation of "homoduplex patches" suggests the presence of some double splices in her "patch" data.

In crosses that probably involved the RecBCD pathway, HERMAN (1965, 1968) and MESELSON (1967) saw reciprocality of homologous recombination between markers on the *E. coli* chromosome and on an *F'* plasmid. The reciprocality was partial in that a recombinant was found segregated with its complementary recombinant about half the time. It seems possible that their observations are akin to those of our λ × λ cross–in a given interaction, complementary recombinants are formed dependent on the genotype of the third participating chromosome (Figure 3). The third chromosome in HERMAN'S AND MESELSON'S experiments could variably be a sister of either the *E. coli* chromosome or the low copy number plasmid that was engaged in the primary event.

THALER*et al.* (1989) demonstrated that RecBC(D^-) protein effects recombination *in vivo* in a manner very like that effected by a wild-type enzyme molecule that has encountered χ. Those results and the results reported here support the view that the recombination-stimulating encounter between χ and the RecBCD enzyme has as its primary consequence the dissociation of the RecD subunit from the enzyme (D. S. THALER, personal communication; I. SIDDIQI, personal communication; CHAUDHURY and SMITH 1984 and AMUNDSEN *et al.* 1986).

We thank ANDREW KUZMINOV, Institute of Cytology and Genetics, Novosibirsk, and SHUMO LIU, Massachusetts Institute of Technology, Cambridge, Massachusetts, for proposing that the previously reported reciprocality of RecBCD-mediated λ recombination was less straightforward than it appeared. ALLAN CAMPBELL will not be surprised. We thank SHUMO LIU for communicating unpublished results. Our laboratory colleagues helped us interpret our experiments. TONY POTEETE and RIK MYERS provided guidance at critical moments. GERRY SMITH, ANDY TAYLOR, ABDUL CHAUDHURY, DON ENNIS, IRA HERSKOWITZ, R. N. RAO and MAURY FOX kindly supplied bacteria or plasmids. RIK MYERS, ICHIZO KOBAYASHI, ANDREW KUZMINOV, JAMES SAWITZKE, RICHARD KOLODNER, DAVID THALER and, especially, GERRY SMITH helped us with the manuscript. We are immensely grateful to SYLVIA CRUMP and the staff of the Molecular Biology Media Room, who reliably provided the glassware and growth media for this project, as they have done for so many others. Financial support was from National Institutes of Health grant GM 33677 and National Science Foundation grant. PCM 8905310. F.W.S. is American Cancer Society Research Professor of Molecular Genetics.

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APPENDIX

In Vivo, the activity of a χ site is reduced but not eliminated by the presence of a χ site to the right of it

When RecBCD enzyme enters λ at the right end and travels leftward, it responds to χ as shown by the resulting increase in the probability of exchange. Exchanges occurring to the left of the χ could all be a consequence of that χ-RecBCD interaction. On the other hand, some of the exchange left of χ could be occurring not as a direct result of the presence of the χ sequence: (1) A RecBCD enzyme molecule that has been activated by χ might return to its ground state as it travels leftward. Such a molecule could again be activated by a χ or χ-like sequence. (2) RecBCD enzyme might sometimes pass χ without responding to it. Such a molecule would be available for activation as it continued its journey left of χ. We have quantitated the ability of χ^+ C to stimulate recombination when it is "shaded" by χ^+D upstream (right) of it. We find that the downstream χ sequence retains activity but that the activity is reduced by the upstream sequence.

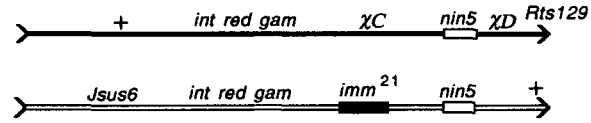

Figure 16.—Crosses to assess the activity of χ^+C in the presence of χ^+D. The upper parent was, respectively, χ^0, χ^+C, χ^+D, or χ^+C χ^+D. When χ^+C, was absent, the upper parent carried the mutation *cII68*, which, like χ^+C is in *cII* and covered by imm^{21}. Each parent was mutant for λ recombination functions by virtue of the mutations *int4*, *red3* and *gam210*. Crosses were in the Su^- strain FS2765, which, incidentally, carries the plasmid pBR325. J^+R^+ recombinants were selected at high temperature on the Su^- *recD* host V218.

To see interactions between χ^+C and χ^+D we conducted crosses like that diagrammed in Figure 16. The χ sequences, when present, were on the *Rts* parent. That phage carried either χ^+C, χ^+D, both χ^+'s or neither χ^+. χ^+C stimulates exchange to its left, in the interval *Jsus6-imm*^{21}. This stimulation is manifested as an increase in the fraction of turbid (cII^+) plaques among selected $J^+ R^+$ recombinants. χ^+D stimulates exchange predominantly in the interval imm^{21} *Rts129*. This stimulation is manifested as an increase in the fraction of J^+R^+ plaques that are clear (χ^+C or *cII68*, both of which are allelic *to* imm^{21}). To test for interaction between χ's, we compute the means of the fractions of plaques that are clear and that are turbid and compare those means to the observed fractions when the *Rts* parent carries both χ sequences.

We conducted the crosses under conditions of free DNA replication (in strain FS2765) and under replication-blocked conditions (in strain FS2766). (The presence of pBR325 in these strains was incidental.) The later conditions are more stringent, since the right end of λ is the only entry point for RecBCD enzyme when replication is blocked (Stahl, Kobayashi and Stahl 1983). In the event, both kinds of crosses showed that χ^+C activity is reduced to about half of its normal value by the presence of χ^+D (Table 6).

Yagil and Shtromas (1985) reported reduction of activity of a χ element in the presence of a χ element upstream (right) of it.

TABLE 6

Activity of χ^+C in the presence of χ^+D

	Free DNA replication				Blocked DNA replication			
	Plaques		Fraction		Plaques		Fraction	
	c	c^+	*c*	c^+	*c*	c^+	*c*	c^+
χ^0	185	605	0.234	0.766	387	1470	0.208	0.792
χ^+C	48	994	0.046	0.954	164	1523	0.097	0.903
χ^+D	498	621	0.445	0.555	1177	409	0.742	0.258
$\chi^+C\ \chi^+D$	350	609	0.365	0.635	944	668	0.586	0.414
$(\chi^+C + \chi^+D)/2$			0.245	0.755			0.420	0.581
$[(\chi^+C + \chi^+D)/2]^a$			0.253	0.747			0.410	0.590

The crosses described in Figure 16 were conducted in FS2765 and FS2766 for free and blocked DNA replication, respectively. The four free replication crosses and the four blocked replication crosses, respectively, were plated for J^+R^+ recombinants at the same dilution for each cross. Resulting plaques were scored as clear (*c*) or turbid (c^+. We quantitate the χ^+C contribution in the presence of χ^+D by seeking the weighting factor that will allow the $c{:}c^+$ ratios of the single χ crosses to average to the value observed for the double χ cross. For free replication, this factor, *f*, can be calculated as $f(0.046) + (1 - f)(0.445) = 0.365$; $f = 0.20$. For blocked replication, $f(0.097) + (1 - f)(0.742) = 0.586$; $f = 0.24$. These values imply that χ^+C is shaded by χ^+D to 0.40 or 0.48 of the activity it has in the absence of χ^+D.

[a] Mean of χ^+C and χ^+D weighted for the respective recombinant phage yields.