Break-Join Recombination in Phage λ

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ABSTRACT

In phage λ, when DNA replication is blocked, recombination mediated by the Red pathway occurs only near the double-chain break site, *cos*, that defines the termini of the virion chromosome. The recombinants initiated by *cos* contain newly synthesized DNA near *cos*, in amount corresponding to a few percent of the length of λ. A restriction enzyme cut delivered to one parent far from *cos* results in elevated recombination near the restriction site. Recombinants induced by this cut have a similarly small amount of DNA synthesis in these replication-blocked crosses. When restriction cuts are introduced in the presence of normal amounts of all of the DNA replication enzymes, many of the resulting recombinants still enjoy, at most, a small amount of DNA synthesis associated with the exchange event. Thus, these experiments fail to support the previously considered possibility that Red-mediated recombination in λ proceeds largely through a break-copy pathway.

Previous work (STAHL *et al.* 1972b) has shown that recombination in phage λ is partially dependent on DNA replication. The degree of dependence is related to distance from the ends of the standard linkage map, so that, in crosses blocked for DNA replication, there is a relative paucity of recombinants in the middle of the map. When replication is allowed, the exchange distribution is more nearly uniform.

The significance of the relationship between high recombination (when DNA replication is blocked) and the end of the linkage map was established in experiments that turn the linkage map inside out (STAHL, KOBAYASHI and STAHL 1982). λ strains were constructed in which *cos*, the region of the λ chromosome that specifies the termini of the packaged chromosome, was translocated from its normal position to the middle of the normal chromosome. Such "inside out" chromosomes are packaged from the translocated *cos*, and have their ends in the region corresponding to the middle of ordinary λ. In these phage, the region of high recombination rate was seen to have been translocated along with *cos*.

Two explanations for the apparent recombinagenicity of *cos* have been considered. In one theory, recombination-initiating events are distributed along λ without regard to *cos*, even among unreplicated chromosomes. Because of a postulated break-copy recombination mechanism, only events initiated near the termini are completed and packaged when replication forks are blocked (but polymerases remain intact) (STAHL *et al.* 1973). In the second theory, *cos* is itself an initiator of recombination (RUSSO 1973). Among unreplicated phage, *cos* is the principal, and perhaps the only, initiator. The act of replication generates other initiators, so that recombination among replicated phages (which may be strictly break-join) is no longer focused at *cos*.

Subsequent experiments supported the latter interpretation without ruling out appropriately adjusted versions of the former (STAHL, KOBAYASHI and STAHL, 1985). It was shown that a functional *cos* site can stimulate break-join recombination even when the parent with which it is paired carries an uncuttable mutant *cos* at that locus. Among *cos* stimulated recombinants, the cuttable *cos* became converted to its uncuttable allele (and the recombinants were packaged via *cos* sites cloned elsewhere in the two parents). Thus, *cos* appeared to be initiating recombination by being a double chain break (DCB) site, more-or-less in the sense of the "double-strand-break repair" model of SZOSTAK *et al.* (1983). This concept led to the proposal that *cos* is the only DCB site among non-replicated phage and that replication introduces other such sites, or their functional equivalents. Most attractive was the possibility that the end of the tail of a rolling circle replicative form could initiate exchange (WILKINS and MISTRY 1974; SKALKA 1977; STAHL, KOBAYASHI and STAHL 1985; THALER, STAHL and STAHL 1987b).

In this paper we demonstrate that a DCB introduced with a restriction endonuclease near the middle of one parent λ chromosome does stimulate break-join recombination in its immediate vicinity. This result supports the view that the usual failure to see recombination in the middle of the λ chromosome in replication-blocked crosses is due to a lack of double

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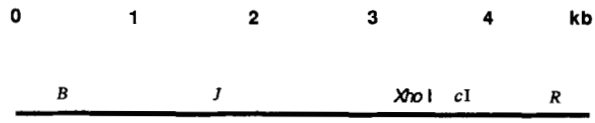

FIGURE 1.—Locations on the λ chromosome of genetic elements employed.

TABLE 1

Bacterial strains and plasmids employed

K12SH-28; Su$^+$ indicator to score *cI vs. cI*$^+$ among Ts$^+$ recombinants; host for heavy stocks (FANGMAN and NOVICK 1966)
FS2769: K12SH-28[pPAORM3.8]; host for heavy, "*Xho*I-modified" stocks
FA77: Su$^-$ derivative of FA22 (FANGMAN and NOVICK, 1968), a *dnaBts* derivative of K12SH-28; host for replication-blocked crosses (MCMILIN and RUSSO 1972)
FS1441 = FA77[pPAORM3.8]; RecA$^+$ host for replication blocked crosses with *Xho*I cutting
FS2594 = FZ14[pPAORM3.8]; RecA$^-$ host for replication blocked crosses with *Xho*I cutting (FZ14 is Su$^-$ *dnaBts22 recA56*) (STAHL *et al.* 1972a)
FS85 = QR47(λ *Jts15 Rsus5*); RecA$^+$ host for repressor crosses
FS86 = QR48(λ *Jts15 Rsus5*); RecA$^-$ host for repressor crosses
FS88 = 594(λ *Jts15 imm434 Rsus5*); Su$^-$ indicator for repressor crosses
pPAORM3.8 encodes the *Pae*R7 restriction system, an isoschizomer of *Xho*I (GINGERAS and BROOKS 1983)

chain ends there. In so doing, it further supports the view that *cos* is a recombinator by virtue of being a DCB site.

Our experiments reveal and quantitate a modest amount of DNA synthesis associated with a *cos*-initiated event and suggest a similar amount of synthesis associated with a centrally located recombination event initiated by an artificially introduced DCB.

Most of our experiments involve λ that is wild type for genes influencing homologous recombination (*red*$^+$*gam*$^+$) recombining in wild type *Escherichia coli* (*rec*$^+$). Thus, they describe the activity of the RecA-assisted Red pathway of λ operating in the presence of Gam-inhibited RecBCD enzyme (for reviews, see SMITH 1983, THALER and STAHL 1988). In some experiments, which will be identified, the host is a *recA* mutant, so that the Red pathway is mediating exchange without help from the RecA protein.

MATERIALS AND METHODS

Phage and bacteria: A map of λ showing features relevant to this work is in Figure 1. Strains of bacteria employed are described in Table 1.

Replication-blocked crosses: Except where noted, crosses were executed in the Su$^-$*dnaBts* host FA77, or a plasmid-carrying derivative thereof, at 40° (MCMILIN and RUSSO 1972). All phages were conditionally defective in the λ DNA replication gene *P* by virtue of the suppressible mutation *Psus80*. Except where noted, phages were *int4*, inactivating λ's site specific recombination system.

Crosses with *in vivo* restriction: In experiments involving cutting of λ's *Xho*I site, the host cell carries the plasmid pPAORM3.8 (GINGERAS and BROOKS 1983), which encodes the *Pae*R7 restriction/modification system, an isoschizomer of *Xho*I. In this paper, both the restriction system and its cut site in λ will be referred to as *Xho*I. Host cell cultures that carry plasmid were grown to 1.5×10^8 cells/ml in ampicillin to hold the plasmid. The cells were removed from the ampicillin and heated at 40° for 10 min. One milliliter of culture was then added to 1 ml of preheated phage mix in which each parent was typically present at 1.0×10^9/ml to give a multiplicity of infection (moi) of 7 of each parent. As in THALER, STAHL and STAHL (1987a), when one parent was cuttable, the uncuttable parent was added 10–15 min before the cuttable one, in order to establish the Red and Gam functions before any DNA cutting occurs. Ninety minutes after infection, the 2-ml infected cultures were lysed with $CHCl_3$ and lysozyme, sometimes after removing unadsorbed phages by centrifugation. The entire volume of each lysate was adjusted to a refractive index of 1.378 with cesium formate and centrifuged in a SW50.1 head at about 30,000 rpm for 16 hr or more. Fractions were collected through a needle hole in the bottom of the tube, usually at two drops per fraction yielding about 75 fractions. Fractions were plaque-assayed on appropriate bacteria as indicated.

Preparation of heavy phage: Density-labeled phage were usually prepared on minimal agar plates in which the glucose was substituted with ^{13}C (99%, in most cases) and the ammonium chloride with ^{15}N (99%). Uncuttable parents were modified by preparation of the density labeled stocks in *E. coli* strain FS2769, a Su$^+$ prototroph carrying pPAORM3.8.

Preparation of radioactive, heavy phage: Heavy phage labeled with ^{32}P or ^{33}P were prepared by thermal induction of the lysogen K12SH-28 (λ *int*$^+$ cI857 *Psus80 Ssus7*)/λ growing in low phosphate (15 mg/liter P) minimal medium. The carbon source was [^{13}C]glucose (~60% ^{13}C) at 2 mg/ml; nitrogen was $^{15}NH_4Cl$ (98% ^{15}N) at 1 mg/ml; ^{32}P or ^{33}P was present as phosphate at a specific activity of 1–2 mCi/mg P. Radioactively labeled phage were purified by equilibrium centrifugation in CsCl. The replication-blocked density-transfer experiment was conducted 10 days after labeling the phage. DNA was released from phage by addition of EDTA (0.01 M) and Sarkosyl (0.1%) and heating for 6 min at 63°. Equilibrium centrifugation of DNA in CsCl was for 40 hr at 38,000 rpm in a SW 50.1 Beckman rotor. Activities of ^{32}P, ^{33}P, and ^3H were determined by scintillation counting (Packard Tri Carb).

DNA extraction for restriction endonuclease digestion: Cells from 100 ml of infected culture were washed several times with chilled nonradioactive medium containing excess phosphate. The final bacterial pellet was resuspended in citrate buffer to which 3×10^{11} nonradioactive phage had been added. Lysis was promoted with $CHCl_3$ and Brij (0.2%). Phage banded in CsCl were dialyzed against 10 mM Tris, 10 mM EDTA, pH 8.0 (TE). Phage DNA was extracted with cold phenol and dialyzed against TE. To separate λ's terminal fragments from each other, endonuclease digests were heated at 65° for 3 min and then quickly chilled before electrophoresis.

RESULTS

***Xho*I can initiate break-join recombination:** λ contains a solitary *Xho*I site, at base pair 33,498, which is 0.69 of the distance from the left to the right end (Figure 1). When one of the two infecting parents carries the *Xho*I site in an unmodified (cuttable) state, *Xho*I endonuclease acting *in vivo* stimulates recombination in that region of λ (THALER, STAHL and STAHL

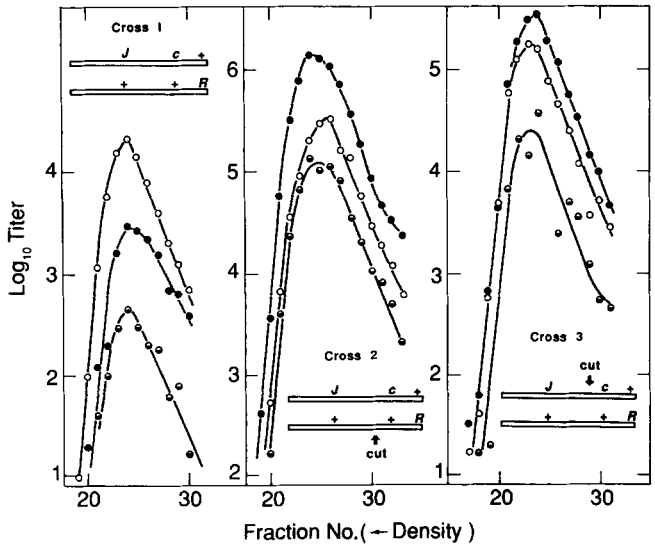

FIGURE 2.—Stimulation of recombination by *Xho*I in replication-blocked crosses. Crosses of *Jts15* c1857 $R^+ \times J^+$ cI^+ *Rts*2 were at restrictive temperature in the *E. coli* K12 strain FS1441, a Su^- *dnaBts22* strain carrying the *Pae*R7 ("*Xho*I") restriction system on a plasmid (pPAORM3.8). Both parents were *Psus80*, so that DNA replication was "double-blocked." In cross 1, both parents were uncuttable by virtue of being *Xho*I-modified. In cross 2, the *Rts* phage was cuttable. In cross 3, the *Jts* cI phage was cuttable. J^+ R^+ recombinants were selected at 40° on the Su^+ indicator K12SH-28, and plaques were scored as clear (cI), turbid (c+) or mottlers (heteroduplexes cI/c+). The data points for each cross, summed according to genotype, are in Table 2. Key: (○) λ J^+ cI^+ R^+ recombinants; (●) λ J^+ cI R^+ recombinants; and (◒) λ J^+ cI/cI^+ R^+ recombinants (heteroduplex at cI).

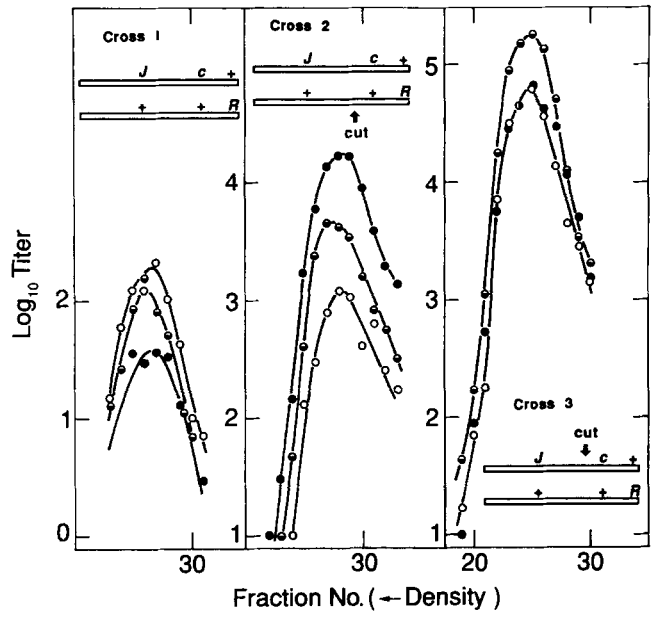

FIGURE 3.—Stimulation of recombination by *Xho*I in $RecA^-$ replication-blocked crosses. Crosses and markers are as in Figure 2. The host strain, FS2594, was *recA56* in addition to being *dnaBts22* and carrying the *Xho*I restriction plasmid. Key as in Figure 2.

1987a). The stimulation can be measured as an increase in the recombinant frequency for a marked interval containing the *Xho*I site relative to the frequency of recombinants in a control interval. In the studies of THALER, STAHL and STAHL (1987a) the absolute frequency of recombinants in the test interval increased. Some of their crosses were conducted under conditions presumed to block DNA replication, with similar results.

We have performed crosses like those of THALER, STAHL and STAHL (1987a) except that both parents were labeled with heavy isotopes of C and N, allowing us to monitor the block on DNA replication. The parents carry conditional lethal mutations in genes *J* and *R*, respectively (Figure 1), so that recombinants can be selected. The *J–R* interval is bisected by a marker at the cI locus, so that recombinants arising in the *J–*cI interval make clear plaques while those arising in the cI–*R* interval make turbid plaques. The *Xho*I site is in the *J–*cI interval. Replication was blocked by the *dnaBts* mutation in the host and *Psus80* in the phages. Three crosses were performed. The parental phages were genotypically the same for each of the three crosses, but they differed as to modification at the *Xho*I site. In cross 1, both parents were modified; in crosses 2 and 3, one or the other parent, respectively, was modified. Since DNA cannot be cut by *Xho*I when it is *Xho*I-modified (and see THALER, STAHL and STAHL 1987a), cross 1 is the control for crosses 2 and 3.

The cross lysates were centrifuged in cesium formate. Each cross yielded a single peak of recombinant phage, demonstrating that replication was well blocked (Figures 2 and 3).

When the host was $RecA^+$ (FS1441), *Xho*I-stimulation of recombination is manifested by a change in the ratio of clear to turbid Ts^+ recombinants (Figure 2). Upon cutting either parent, the relative frequency of clear to turbid recombinants increases, implying a relative increase in recombination in the *J–*cI interval, which contains the *Xho*I site. In both crosses involving cutting, the relative frequencies of mottled (cI/cI^+ heteroduplex) to turbid (cI^+) plaques are increased by cutting, as well. The former observation needs no interpretation; we will interpret the latter observation in DISCUSSION.

The parental phage mixes used in the $RecA^+$ crosses above were used to infect the $RecA^-$ strain, FS2594. In these crosses (Figure 3), the Red pathway is operating without assistance from RecA protein. The results of the crosses are qualitatively like those of the $RecA^+$ crosses. The *Xho*I cut shifts recombination into the interval containing the cut site, and, among J^+R^+ recombinant particles, cI/cI^+ heteroduplexes are increased relative to cI^+ homoduplexes. In cross 3 heteroduplex particles are an absolute majority of all recombinants, a result not seen in the corresponding $RecA^+$ cross (Figure 2, cross 3). A high heteroduplex frequency is seen in cross 2, as well, where cI/cI^+ heteroduplexes outnumber cI^+ recombinants, in con-

TABLE 2

***Xho*I stimulated recombination in replication-blocked crosses**

Crosses	Fraction of λ recombinant in interval			
	J-*c*I	*c*I-*R*	*c*I-*c*I$^+$ (hets)	hets/*c*I-*R*
RecA$^+$				
Cross 1				
Expt. 1	0.15	0.83	0.02	0.02
Expt. 2	0.16	0.83	0.02	0.02
Cross 2				
Expt. 1	0.76	0.17	0.07	0.4
Expt. 2	0.75	0.17	0.08	0.5
Cross 3				
Expt. 1	0.63	0.33	0.04	0.1
Expt. 2	0.38	0.55	0.08	0.2
RecA$^-$				
Cross 1				
Expt. 1	0.12	0.55	0.34	0.6
Expt. 2	0.14	0.77	0.09	0.1
Cross 2				
Expt. 1	0.76	0.05	0.19	3.8
Expt. 2	0.76	0.06	0.18	3.0
Cross 3				
Expt. 1	0.22	0.19	0.59	3.1
Expt. 2	0.24	0.16	0.61	3.8

Experiment 1 is from Figures 2 and 3. In cross 1, neither parent is cut. In cross 2, the *Rts* parent is cut. In cross 3, the *Jts* parent is cut.

trast to the corresponding RecA$^+$ crosses (Figure 2, cross 2).

The set of six crosses was repeated with similar results. For the two sets of crosses the fractions of J^+R^+ that are *c*I, *c*I$^+$, or *c*I/*c*I$^+$ heteroduplex, respectively, are reported in Table 2.

The progeny λ particles from RecA$^+$ double-blocked infections contain various small amounts of newly synthesized DNA: Two populations of density-labeled λ *Psus80* were prepared, one in ^{32}P-containing medium and one in ^{33}P-containing medium. The populations were calibrated for their relative levels of DNA density label by centrifugation of DNA extracted from the particles (Figure 4A). The ^{32}P-labeled phage was purified and then passed through a double-block cycle at a moi of 10 in strain FA77, and the progeny was centrifuged in cesium chloride. Plaque-assay of the fractions showed that the infection gave a unimodal density-distribution of progeny phages, characteristic of double-block crosses (Figure 4B). Unadsorbed phage (not shown) are well separated from the progeny peak by virtue of their heavy-labeled protein coats. Phage particles from the heavy flank of the progeny peak, from the modal fraction of the peak, and from fractions on the light flank, respectively, were combined with the heavy ^{33}P λ and with light ^{3}H-labeled *E. coli* DNA, and DNA was released from the phage particles. The three DNA mixtures were centrifuged in CsCl (Figure 4, C–E). DNA from the phage particles in the successively lighter fractions is seen to be progressively lighter than the ^{33}P reference. The density shifts imply new DNA synthesis to the extent of 1.6% and 4.5% of the λ chromosome in the modal and lighter fractions, respectively.

Under replication-blocked conditions, recombination is prerequisite to chromosome packaging, presumably because it provides the sole route to concatemer formation (STAHL *et al.* 1972a). Thus, all of the chromosomes in the lysate of Figure 4B have enjoyed an exchange during their sojourn in the replication-blocked host cell. Since the recombination under these Red$^+$ Gam$^+$ RecA$^+$ conditions is primarily (or exclusively) *cos*-initiated, most, at least, of the chromosomes in Figure 4B have enjoyed an exchange near *cos*. For the Red system operating in double-blocked RecA$^-$ cells, the amount of new DNA in individual progeny λ particles is correlated inversely with the probability of heterozygosity for a marker near the right end of the chromosome (STAHL and STAHL 1986). That correlation was taken to imply that *cos*-initiated, Red-mediated recombination was accompanied by variable amounts of DNA synthesis at the site of the exchange. In the following section we show that the incorporated label in the Red$^+$ RecA$^+$ double block progeny is concentrated near the termini, where most of the *cos*-initiated exchange occurs.

The ^{32}P incorporated in the λ progeny of the Red$^+$ RecA$^+$ double-blocked infection is concentrated in the terminal restriction fragments: FA77 was grown at 26° to 1.5 × 10^8/ml in Tris-maltose (HERSHEY 1955) plus 0.2% Bacto-Peptone (as sole source of nonradioactive phosphorus). The 100-ml culture was oxygenated at 39° for 10 min and then infected with nonradioactive ^{12}C ^{14}N λ *int*$^+$ cI857 *Psus80 Ssus7* at a moi of 20. At this time, glucose was added to a final concentration of 0.1%, and 5 mCi ^{32}P were added as carrier-free phosphate. Progeny phage were collected at 90 min, at which time the yield is about one phage particle per cell, and purified as described in MATERIALS AND METHODS. DNA was extracted with cold phenol and digested with *Eco*RI or *Hin*dIII as described in MATERIALS AND METHODS. The resulting restriction fragments were sorted by gel electrophoresis. The tube gel was stained to identify bands of restriction fragments, and slices 2 mm thick were scintillation counted. Results are in Table 3. They show that the specific activity of the terminal restriction fragments exceeds that of each of the other fragments. These results demonstrate that the new DNA found in the progeny of a double-blocked Red$^+$ RecA$^+$ infection is located primarily in the regions of the chromosome where recombination is maximal as a result of *cos*-initiated exchange.

DNA synthesis in a *Xho*I-induced exchange: It is

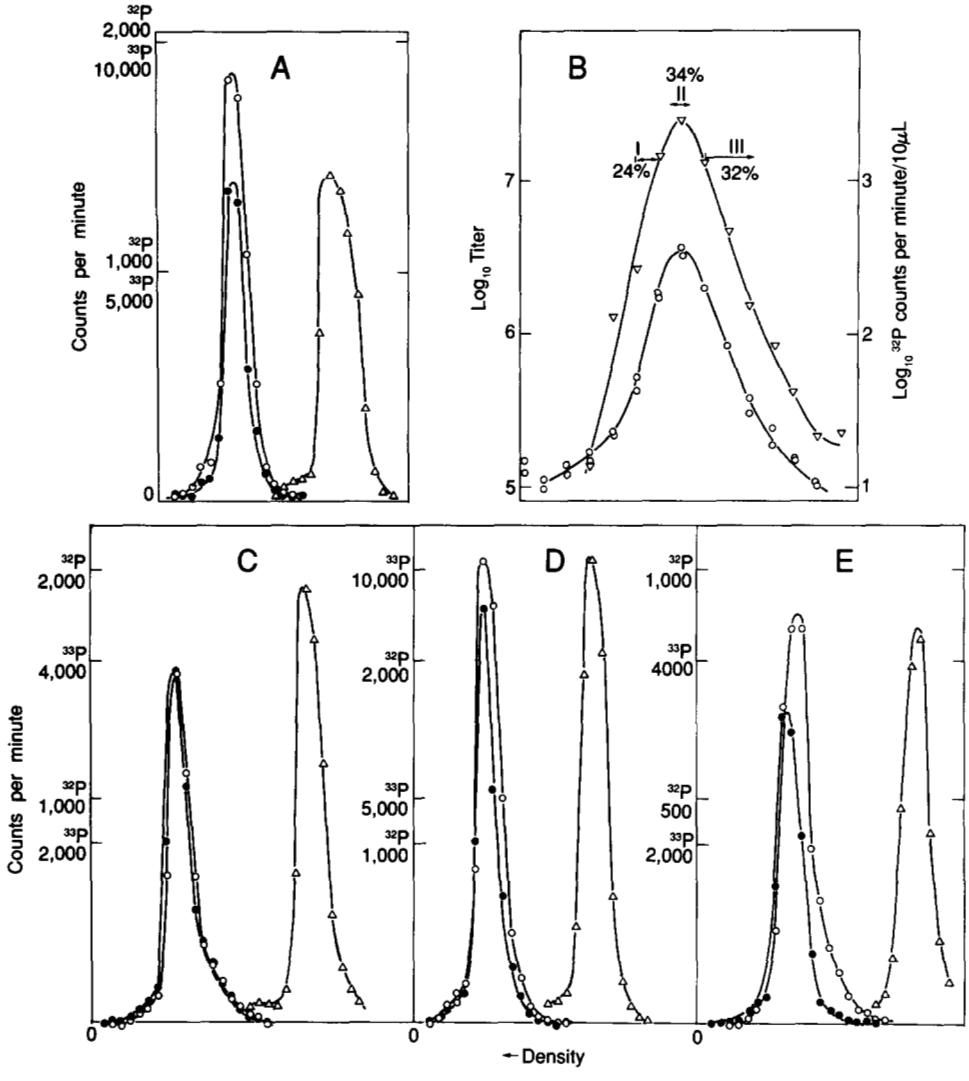

FIGURE 4.—New DNA in chromosomes rendered packageable by a *cos*-stimulated exchange. (A) λ *c*I857 *Psus80 Ssus7* phages were grown in heavy medium (^{13}C and ^{15}N) labeled with ^{32}P and ^{33}P, respectively. Samples of each labeled phage stock were combined and DNA was extracted. The DNA was centrifuged for 40 hr at 38,000 rpm in CsCl along with ^{3}H-labeled light *E. coli* DNA. Fractions were collected through a needle hole in the bottom of the tube, dried, and counted in three-channel scintillation counter. (B) The density-labeled, ^{32}P-labeled phage stock infected the *dnaBts* strain FA77 at a moi of 20 at 40°. Progeny particles were centrifuged in cesium formate. Fractions were collected and plaque-assayed. Samples (I–III) containing 24%, 34% and 32% of the phage, respectively, from the heavy, middle, and light portions of the peak were identified as indicated. (C–E) DNA isolated from each of identified samples (I–III) in (B) was centrifuged in CsCl with ^{33}P-labeled heavy DNA and ^{3}H-labeled light DNA as density references. The amount of newly synthesized DNA in the DNA from the passaged, ^{32}P-labeled phage is estimated from the shifts in density of the DNA from the fully heavy (^{33}P) position to the fully light (^{3}H) position. Key: (○) ^{32}P counts; (●) ^{33}P counts; (△) ^{3}H counts; and (▽) phage titer.

TABLE 3

Locations of ^{32}P incorporated in Red$^+$ RecA$^+$ double-block infection

Fragment	Position (kb)	Percent length	cpm	Percent cpm	Percent cpm/ percent length
*Eco*RI					
A	0–21.2	44.5	1444	70.3	1.58
B	21.2–26.1	9.8	49	2.4	0.24
C	26.1–31.7	11.3	52	2.5	0.22
D	31.7–39.2	15.4	110	5.4	0.35
E	39.2–45.0	12.1	153	7.4	0.61
F	45.0–48.5	6.9	247	12.0	1.74
*Hin*dIII					
A	0–23.1	47.6	850	59.6	1.25
B	23.1–25.2	4.3	20	1.4	0.33
C	25.2–27.5	4.7	27	1.9	0.40
D	27.5–36.9	19.4	200	14.0	0.7
E	36.9–37.5	1.2			
F	37.5–44.1	13.6	170	11.9	0.88
G	44.1–48.5	9.1	160	11.2	1.23

shown in a later section that most of the recombinants in a λ*Bts* × λ*Rts* replication-blocked, *Xho*I-stimulated cross are *Xho*I-initiated, rather than *cos*-initiated. Thus, DNA synthesis associated with a *Xho*I-initiated exchange can be estimated by comparing the density of a population of *Xho*I-initiated recombinants with a control, *cos*-initiated, population (Figure 5A). The *cos*-initiated recombinants were Ts$^+$ particles from a replication-blocked cross of *Bts* × *Rts* in which both parents were *Xho*I-modified. In the cross to obtain *Xho*I-initiated recombinants, the *Rts* parent was unmodified. Both parents were *c*I in the *cos* cross, while both were *c*I$^+$ in the *Xho*I cross. The cross lysates, obtained from infection of aliquots of the same culture, were combined and centrifuged with an unlabeled phage of distinguishable genotype added as density reference (Figure 5A). To assess the relative degree of density label in the two sets of phage stocks used in these crosses, the two sets of stocks were used as parents for crosses conducted in the absence of *Xho*I plasmid. Both sets of recombinant progeny in these crosses are packaged consequent to a *cos*-initiated exchange (Figure 5B). The data show that recom-

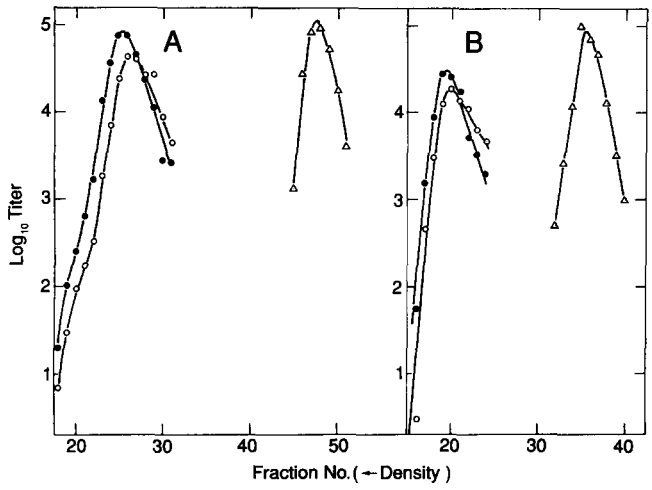

FIGURE 5.—New DNA in chromosomes that have enjoyed a *Xho*I-stimulated exchange. (A) Density-shift associated with a *Xho*I-initiated exchange. Heavy modified λ *BtsI* cI^+ R^+ was crossed with heavy unmodified λ B^+ cI^+ *Rts129* in FS1441. Part of the resulting lysate, in which most of the B^+ R^+ particles had enjoyed a *Xho*I-initiated exchange, was spun with the lysate from a cross on the same host of heavy modified λ *Bts1* *cI* R^+ × heavy modified λ B^+ *cI* *Rts129*. The recombinants in the latter cross are all *cos*-initiated. The gradient was plated at high temperature on a *Su*II$^+$ host to score clear and turbid B^+ R^+ recombinants. Light density reference phage (λ *cI*857 *Ssus7*) were added to the gradient and scored on a SuIII host at temperature restrictive for the *Bts* and *Rts* parental genotype phages. (B) Similarity of label of parental phage stocks. The pairs of phage stocks used in (A) were tested for equality of DNA density label by crossing them in the same pairwise combinations used in (A). The host used for these crosses was FA77, the same as that used in (A) except lacking the *Xho*I plasmid. Thus, exchanges generating the B^+ R^+ recombinants are *cos*-initiated for both crosses. Key: (Δ) Light density reference λ *cI*857 *Ssus7*; (●) B^+ *cI* R^+; and (○) B^+ cI^+ R^+.

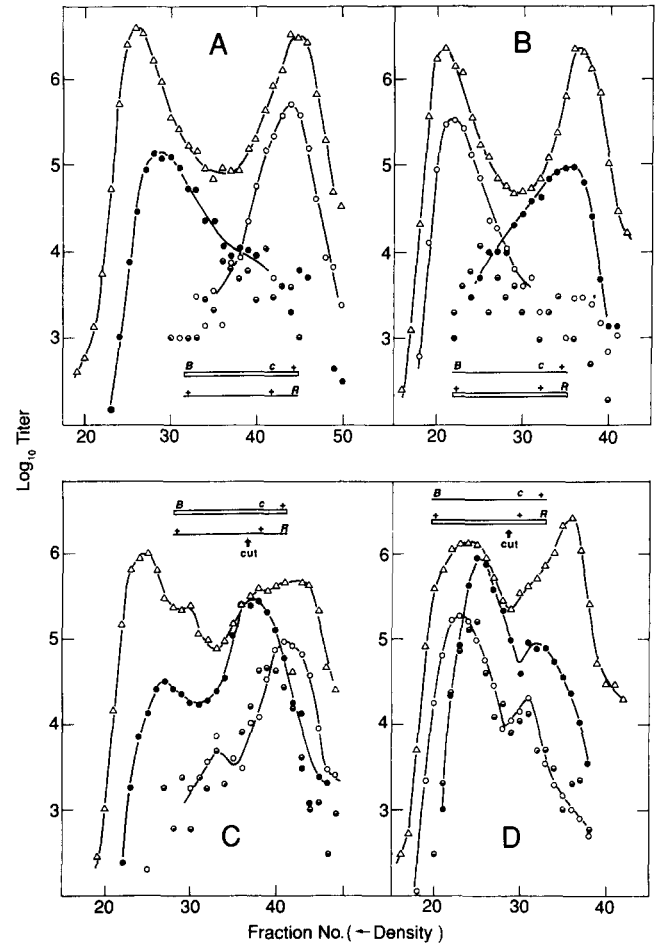

FIGURE 6.—Distribution of exchanges in *Xho*I-cut chromosomes. Replication was blocked by *dnaBts22* in the Su$^-$ host FS1441 and by *Psus80* in all parental phages. Lysates were spun in cesium formate, and density fractions were assayed for total phage on K12SH-28 at 32° and for B^+ R^+ recombinants on the same strain at 40°. (A) Heavy λ *Bts1* *cI26* R^+ × light λ B^+ cI^+ *Rts129* with both parents modified (no *Xho*I cutting). (B) Light λ *Bts1cI* *26* R^+ × heavy λ B^+ cI^+ *Rts129* (both modified). (C) Heavy, modified λ *Bts1* *cI26* R^+ × light, unmodified (*Xho*I-cuttable) λ B^+ cI^+ *Rts129*. (D) Light, modified λ *Bts1* *cI26* R^+ × heavy, unmodified λ B^+ cI^+ *Rts129*. Key: (Δ) Total λ; (○) λ B^+ cI^+ R^+ recombinants; (●) λ B^+ *cI* R^+ recombinants; and (◒) λ B^+ cI/cI^+ R^+ recombinants (heteroduplex at *cI*).

binants packaged following a *Xho*I-initiated exchange have a density distribution like those that have been packaged following a *cos*-initiated exchange, implying that *Xho*I-initiated exchange, also, involves only a small amount of DNA synthesis in double block crosses.

A *Xho*I cut can overcome the lack of recombinants of intermediate density in a heavy × light cross: The extent of DNA synthesis accompanying a *Xho*I-initiated event, in these replication-blocked conditions, is small compared to the length of λ. Therefore, if the heteroduplex segments associated with *Xho*I events are not too long and if degradation from the double chain cut is not too extensive and variable, a *Xho*I-initiated exchange will produce a discrete density peak in a replication-blocked "profile" cross between a heavy and a light parent.

Our replication-blocked crosses were of the following form. Parents carried *ts* markers in the subterminal genes *B* and *R*, respectively (Figure 1). The *Bts* parent carries the marker *cI*857. One parent is heavy and the other is light. *Bts1* substitutes for the *Ats14* marker used in previous papers. As compared to *Bts1* and to the several *Asus* markers examined, *Ats14* used as a marker reduces recombination near λ's left end in replication-blocked Red$^+$ RecA$^+$ crosses (J. M. CRASEMANN, personal communication).

In the control crosses (Figure 6, A and B), both parents were modified, so that there was no cutting by *Xho*I. In Figure 6A, the *Bts* parent was heavy, while in Figure 6B, the *Rts* parent was heavy. The nonuniformity of exchange in replication-blocked crosses (STAHL *et al.* 1974) is manifested in two features of the distributions in Figures 6A and B. (a) Ts$^+$ recombinants are bimodally distributed, with maxima indicating relatively high rates of exchange near the termini (*i.e.*, near *cos*). (b) Most of the total phage fall into the heavy or the light peak. Since all phage must have dimerized via recombination (STAHL *et al.*

1972a), and half the recombination acts will be between phage of opposite parental genotype (VISCONTI and DELBRUCK 1953), a uniform exchange distribution should result in about one-half of the particles having densities that are distributed uniformly between fully heavy and fully light. Since particles of intermediate density are rare, we can again deduce that most of the exchanges occur near the chromosome tips, many of them outside the marked interval (*B–R*).

In the experimental crosses, Figure 6C corresponds to Figure 6A, except that the *Rts* parent is unmodified, while Figure 6D corresponds to Figure 6B, with the *Rts* parent unmodified. Thus, in Figure 6C the light parent is cut, while in Figure 6D the heavy parent is cut.

Comparing Figure 6C with its control, Figure 6A, we see distributions of Ts^+ recombinants that are essentially superimposable except for the peak composed predominantly of clear Ts^+ recombinants whose density indicates that they are the result of exchange near the *Xho*I site. The same relationship holds between Figure 6, D and B, except for the inversion of these distributions relative to Figure 6, C and A (due to inversion of the density label between the two parental phages). Thus, to a first approximation the results are independent of whether the parent that is cut is heavy or light, as expected from our observation that there is not much DNA synthesis associated with a *Xho*I-initiated event.

The curves involving *Xho*I cutting add a peak of B^+ R^+ recombinants without seriously altering the relative amounts of *cos*-initiated exchanges at the left and right ends leading to B^+ R^+ recombinants. This feature of the distributions suggests that B^+ R^+ recombinants manifesting a terminal exchange are primarily "background," arising by exchanges between the uncuttable parent and a cuttable parent that happened to escape restriction.

A *Xho*I cut stimulates break-join recombination even when DNA replication forks are unimpeded: The experiments described above demonstrate that a *Xho*I restriction cut delivered to one parent in a λ × λ cross can stimulate break-join recombination. Those crosses were conducted under conditions that strongly block DNA replication by depriving the infected cells of the *E. coli dnaB* and the λ*P* gene products, so they fail to address the possibility that recombination stimulated by a double chain break would proceed by break-copy, if that route were available. To assess this possibility, we conducted *Xho*I-stimulated crosses in which replication enzymes are fully functional.

λ repressor impedes DNA replication even in the presence of all the replication enzymes. This impedance is due to a requirement for transcription across λ's origin of replication for maximum "activation" of

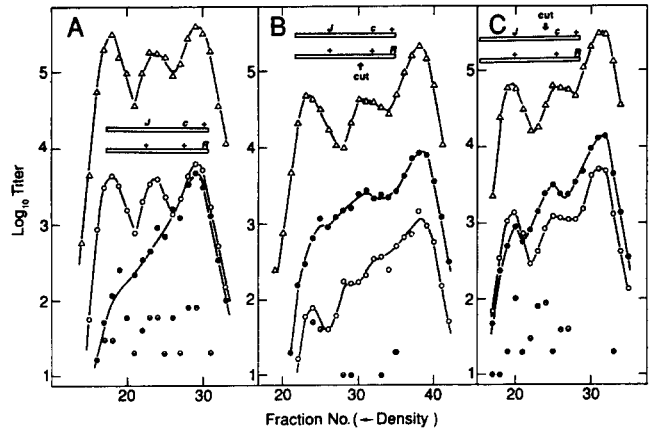

FIGURE 7.—RecA$^+$ repressor crosses. Heavy λ *Jts15 cI*857 R^+ were crossed with heavy λ J^+ cI^+ *Rsus5* in the homoimmune lysogen QR47 (λ *Jts15 Rsus5*) in the presence of preinfecting heteroimmune helper phage *Jts15 imm434 Rsus5*. J^+ R^+ recombinants were plated selectively on the Su^- lysogen 594 (*Jts15 imm434 Rsus5*) at 42° and scored as *cI*857, cI^+, or cI/cI^+ heteroduplexes by inspection of the plaques. (A) Both parents uncuttable (*Xho*I-modified). (B) J^+ cI^+ *Rsus5* parent cuttable. (C) *Jts15 cI*857 R^+ parent cuttable. Key:

	Mean fraction number		
	A	B	C
○ λ J^+ cI^+ R^+ recombinants	24.4	35.7	28.4
● λ J^+ *cI* R^+ recombinants	27.8	35.1	29.4
◒ λ J^+ cI/cI^+ R^+ recombinants			
Δ Total λ imm^λ			

ori [see FURTH and WICKNER (1983) for review]. Previous work (*e.g.*, STAHL and STAHL 1971; STAHL *et al.* 1972a) has exploited this cis-acting effect of λ repressor to limit the amount of DNA replication of the two infecting λ phage in a λ × λ lytic cycle cross. Conventional wisdom (DOVE, INOKUCHI and STEVENS 1971) says that any replication that might be initiated in an *ori*-independent fashion will proceed unimpeded in such a cross. Thus, by inhibiting *ori*-dependent replication we may reveal *ori*-independent replication initiated by a *Xho*I-stimulated recombination event.

The crosses were *Jts15* *cI*857 R^+ × J^+ cI^+ *Rsus5* conducted in bacterial strains that carried λ prophage *Jts15 Rsus5*. Both of the parental phage stocks were heavy-labeled. Repressor made by the resident prophage provides a partial block to *ori*-dependent replication. Since repressor blocks transcription of λ genes, a heteroimmune "helper" phage is added to provide Red and Gam recombination functions, O and P replication functions, and DNA packaging proteins. The imm^{434} helper phage has the genotype *Jts15 Rsus5* so that it cannot contribute to the formation of J^+ R^+ recombinants. The helper phage was uncuttable by *Xho*I and, to ensure the timely presence of Red and Gam functions, was added to the bacteria 12 min before the imm^λ phages. All phages in this cross, as in the others, are Int^-, so that recombination in the *J–cI* interval has no contribution from that site-specific system. Both RecA$^+$ (Figure 7) and RecA$^-$ (Figure 8)

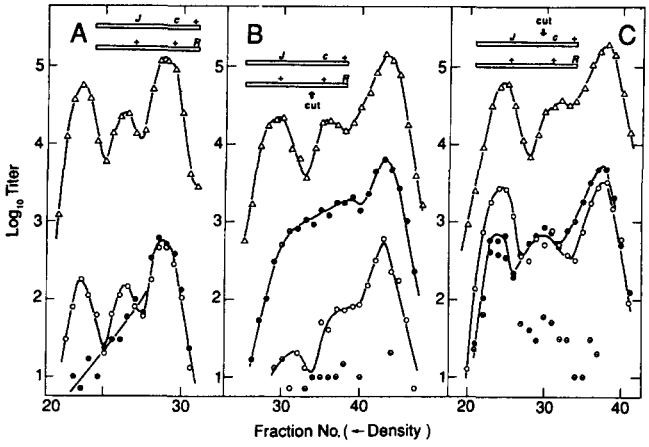

FIGURE 8.—RecA⁻ repressor crosses. As in Figure 7 except that the host cell was QR48 (λ *Jts15 Rsus5*). Key:

	Mean fraction number		
	A	B	C
○ λ J^+ cI⁺ R^+ recombinants	24.9	41.6	31.1
● λ J^+ cI R^+ recombinants	26.9	40.3	34.7
◒ λ J^+ cI/cI⁺ R^+ recombinants			
Δ Total λ imm^λ			

crosses were performed at 37°, which is permissive for *Jts15*.

Figures 7A and 8A present control data, in which neither of the infecting imm^λ phages is *Xho*I-cuttable. As seen in previous work (STAHL and STAHL 1971), this protocol limits DNA replication so that unreplicated chromosomes make up about one quarter of the yield of imm^λ phage. Recombinants in the *c*I–*R* interval (turbid plaques), at the right end of λ, have enjoyed about as many rounds of replication as have the total imm^λ phage. Recombinants in the *J*–*c*I interval (clear plaques), on the other hand, are rare in the fully heavy peak. Only in the fully light peak are the two recombinant types found in relative amounts that approximate the relative physical lengths of the intervals involved. This differential association between DNA synthesis and recombination in the two marked intervals is quantitated by comparison of the average positions in the density gradient of the two classes of recombinant particles. Recombinants in the *J*–*c*I interval are lighter on the average than recombinants in the *c*I–*R* interval (Figures 7A and 8A).

Experiments in the sections above, in which replication enzymes are missing, argue that failure to find fully heavy *J*–*c*I recombinants in double block crosses is due to the lack of double chain ends there. In the experiments of this section, we can anticipate a similar lack of spontaneous initiations among unreplicated chromosomes. We can provide those initiating events with *Xho*I, however, and note whether the resulting recombinants are present in the heavy peak. If an exchange occurring in the presence of the replication enzymes invariably initiates a replication fork, *Xho*I-initiated recombinants in the *J*–*c*I interval may be gone from the fully heavy peak. Crosses in which one or the other of the infecting imm^λ phages is cuttable are in Figures 7, B and C, and 8, B and C.

In Figures 7B and 8B, the *Rsus5* parent was cuttable. In all density peaks, including the fully heavy one, the J^+ R^+ recombinants now arise primarily in the interval, *J*–*c*I, that contains the *Xho*I cut site. Because restriction involves a competition between modification and cutting, it is likely that those heavy, cuttable phages that are cut are cut promptly upon infection of the restricting host cell. Since λ replication is dependent on supercoiling (for review, see FURTH and WICKNER 1983), the chromosomes linearized by restriction are probably not able to replicate. We surmise, therefore, that the *Xho*I stimulated recombinants in the light peak replicated after the *Xho*I-initiated exchange event. The *J*–*c*I interval recombinants, instead of having an average density less than that of the *c*I–*R* interval recombinants, as they did in the control, now have an average density that is slightly greater than that of the *c*I–*R* recombinants. This argues that *Xho*I cutting has provided an initiation structure for break-join recombination that was previously provided by replication. In the *c*I–*R*, control interval, where most initiation is by *cos* cutting, some initiation structures are probably provided by replication. Thus, these density distributions fail to support the possibility that recombinants arising far from *cos*, in the *J*–*c*I interval, can initiate DNA synthesis more extensive than that reported for the double-block crosses described above. The smeared nature of the density distributions, especially of the recombinants arising in the *J*–*c*I interval, which contains the *Xho*I site, could imply extensive DNA synthesis occurring in some acts of exchange. On the other hand, the smeared recombinant density distribution might merely reflect break-join exchanges, near the middle of the chromosome, between heavy restricted chromosomes and chromosomes of the nonrestricted parent that had replicated prior to exchange. The latter view seems the more consistent with our other observations.

In Figures 7C and 8C, the *Jts* cI parent was cuttable. Again, recombination in the *J*–*c*I interval is increased relative to that in the *c*I–*R* interval, and the increase is seen in all of the density classes. As in the double block crosses, the increase in *J*–*c*I interval recombinants relative to the *c*I–*R* interval recombinants is less than in crosses in which the *Rsus* parent was cut. As proposed above, this difference reflects the opportunity for cuts in the *Jts* cI parent to stimulate J^+ R^+ recombinant formation in both the cut interval and the *c*I–*R* interval to the right of the cut. Notably, the induced recombinants are well represented in the fully heavy peak, again testifying that recombinants induced by a double chain break are often completed

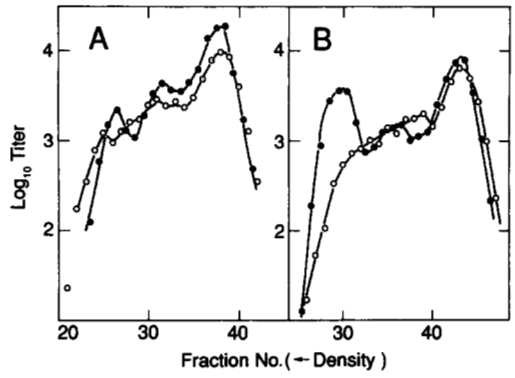

FIGURE 9.—Total J^+ R^+ recombinant density distributions from repressor crosses with one *Xho*I-cuttable parent. (A) RecA$^+$ data of Figure 7, B and C. (B) RecA$^-$ data of Figure 8, B and C. Key: (○) J^+ cI^+ *Rsus5* parent cuttable and (●) *Jts15* *c*I857 R$^+$ parent cuttable.

in a break-join manner even when all the enzymes required for DNA replication are present in the infected cell.

The shapes of the density distributions of total J^+ R^+ recombinants (Figure 9, A and B) depend on which of the two parents was cut. Cutting the *J c*I R^+ parent yields J^+ R^+ recombinants with modal densities corresponding roughly to heavy, half heavy, and light. Cutting the *R* parent, however, gives J^+ R^+ recombinants in which the two heavier modes are not resolved. This difference is compatible with the view that some of the recombinants arise between restricted, heavy chromosomes and chromosomes of the nonrestricted parent that have replicated. When the *J c*I R^+ parent is cut, cut-stimulated J^+ R^+ recombinants can arise by exchange to the right of the cut, near the right end of the chromosome. The resulting recombinant will have a density not very different from that of the nonrestricted parental chromosome with which the cut fragment bearing the R^+ gene interacts. On the other hand, when the *R* parent is cut, J^+ R^+ recombinants will tend to arise by exchange to the left of the cut, near the middle of the λ chromosome. Exchanges between a restricted heavy *R* chromosome and a half-heavy, nonrestricted *J c*I chromosome will create a recombinant that is ~¾ heavy. These recombinants will tend to obscure the heavy and half heavy modes. Thus, the dependence of the density distributions on the genotype of the cut parent is understandable in terms of break-join recombination.

DISCUSSION

Our experiments provide a graphic demonstration that a double chain break in one parent can initiate a break-join exchange with a homologue. They support the view (STAHL, KOBAYASHI and STAHL 1985) that the relative shortage of exchanges in the middle of λ when replication is blocked is due to the lack of initiation events there. They give no reason to doubt that all recombination in the Red pathway is normally

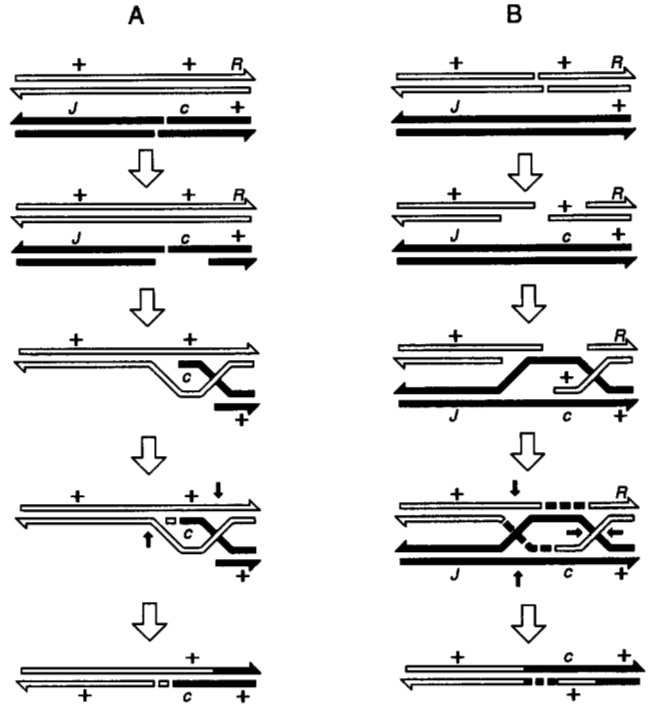

FIGURE 10.—Models for DCB-induced recombination. Broken lines indicate DNA synthesis primed by a 3′ OH end in a recombinational intermediate. Arrows indicate the points of cutting that resolve the postulated intermediates to give the indicated products. (A) One-ended, nonreciprocal "crossing over" (STAHL, KOBAYASHI and STAHL 1985). Compare with cross 2 of Figures 2 and 3. (B) Two-ended, reciprocal crossing over (SZOSTAK *et al.* 1983). In the crosses reported here, the participating right and left fragments need not come from the same cut phage chromosome. Compare with cross 3 of Figures 2 and 3.

initiated at double chain ends; these ends may be provided as the tails of rolling circles when replication is allowed. The documented similarity between *Xho*I-initiated events and unpaired *cos*-initiated events in Red$^+$ RecA$^-$ crosses supports the view that *cos* promotes exchange by virtue of being a DCB site. (Comparable experiments for *cos*-initiated recombination in Red$^+$ RecA$^+$ crosses have not yielded critical data.)

In the double block crosses of Figures 2 and 3, *c*I hets are increased in frequency relative to recombinants in the *c*I–*R* interval in response to cutting. This increase has different implications for the two crosses. For cross 3, in which the cut parent is *J*–*c*I (Figures 2 and 3), the increase in *c*I heteroduplexes (relative to cI^+) implies, simply, that the *Xho*I-induced splice that generates the J^+ R^+ recombinants often laps the *c*I857 site, 4.2 kb to the right of the *Xho*I cut. (The observed frequency of 60% heteroduplexes among J^+ R^+ recombinants in cross 3 of Figure 3 argues that we are failing to detect not more than 40% of the heteroduplexes formed. Thus, mismatch correction or inefficiencies in detection of mottled plaques are unlikely to be intruding seriously in these analyses.) These recombinants could arise by a nonreciprocal break-join event (Figure 10A), a demonstrated route to

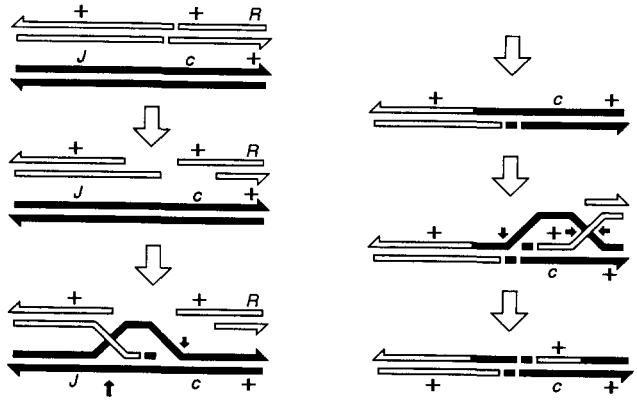

FIGURE 11.—A succession of one-ended events (RESNICK, 1976) can allow both fragments produced by a DCB to contribute to the same crossover chromosome. In the crosses reported here, the participating right and left fragments need not come from the same cut phage chromosome. Symbols as in Figure 10. Compare with cross 3 of Figures 2 and 3.

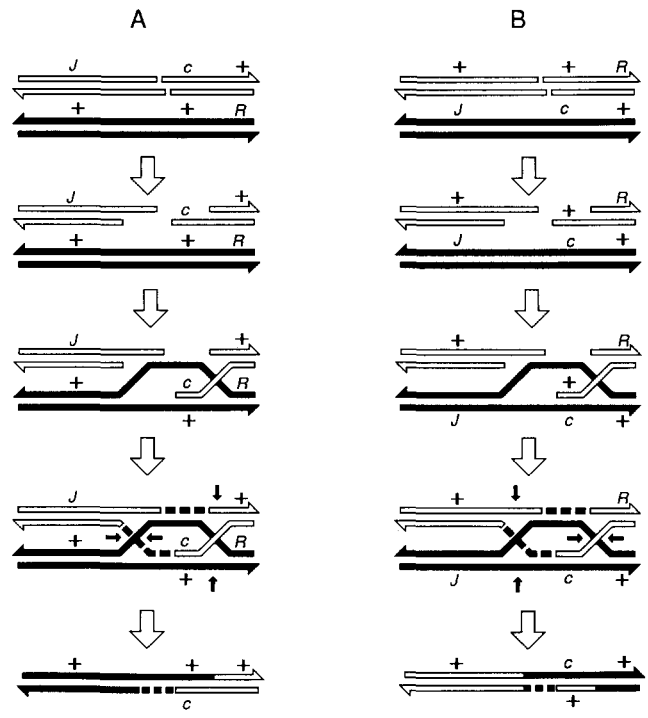

FIGURE 12.—Formation of a symmetric (SZOSTAK *et al.* 1983) intermediate predicts that the frequency of *c*I/*c*I$^+$ heteroduplexes among *Xho*I-initiated J^+ R^+ recombinants is independent of which parent is cut by *Xho*I. (A) Cutting the *J c*I R^+ parent, followed by single-chain degradation rightwards past the *c*I marker, results in the symmetric intermediate containing two Holliday junctions. There are two equally probable ways of resolving this structure to give J^+ R^+ recombinants. Cutting the intermediate horizontally at the left junction and vertically at the right one generates a J^+ R^+ recombinant that is heteroduplex at *c*I. (B) Cutting the J^+ *c*I$^+$ *R* parent followed by degradation leads to a symmetric structure like that in (A). Resolution of this structure by cutting vertically at the left junction and horizontally at the right one gives a J^+ R^+ recombinant that is heteroduplex at *c*I.

recombinant formation by Red, at least in RecA$^-$ cells (STAHL and STAHL 1985). In cross 2 (Figures 2 and 3), however, the increase in heteroduplexes is more conveniently understood in terms of the "double-strand-break repair" model of SZOSTAK *et al.* (1983). The left fragment must contribute the J^+ marker, but cannot contribute the *c*I$^+$ marker found in the heteroduplex. Only the right fragment can contribute that. Therefore, the *Xho*I-induced formation of *c*I/*c*I$^+$ heteroduplexes suggests some invasions of the same uncut homologue by the two fragments followed by resolution of the resulting intermediate so as to segregate the heteroduplex region with the J^+ marker (Figure 10B). Although the model of SZOSTAK *et al.* (1983) deals efficiently with these data, other DCB models might do as well. For instance, RESNICK (1976) proposed that DCBR can involve independent, successive invasions of the same homologue by the DCB ends. A sequence of invasions that could generate the observed heteroduplexes is diagrammed in Figure 11.

In RESULTS, we proposed that the light *Xho*I-initiated recombinants are a result of postrecombinational replication (Figures 7 and 8). Replication probably requires supercoiled DNA, which seems to imply that the cut chromosome interacted with an uncut chromosome to generate a covalently closed, dimeric circle. By this argument, those recombinants that replicated arose by "double-strand-break repair"—both ends invaded the same intact homolog, allowing reciprocal exchange to accompany the repair. The ability of Red to promote "double-strand-break repair" has been argued from studies on recombination between inverted repeats in a plasmid growing in the presence of Red functions (TAKAHASHI and KOBAYASHI 1990).

Quantitative features of our *Xho*I-stimulated RecA$^-$ crosses depend strongly on which of the two parents is cut (*cf.* crosses 2 and 3 in Figures 5 and 6). The *c*I/*c*I$^+$ heteroduplex frequencies in those two crosses differ. Using a different set of markers, THALER, STAHL and STAHL (1987a) noted a similar change in heteroduplex frequency in Red$^+$ RecA$^+$ crosses. Those authors argued that such differences mean that many recombinants arise by a route that does not involve a symmetric intermediate (see Figure 12 for the argument). Thus, the "double-strand-break repair" model of SZOSTAK *et al.* (1983) does not fully account for the properties of Red-mediated, double chain break-induced recombination in either RecA$^+$ or RecA$^-$ cells. Is the synaptonemal complex of recombining (meiotic) eukaryotic cells a device to ensure that the two ends created by a double chain break will always invade the same homolog?

Among J^+ R^+ recombinants, *c*I/*c*I$^+$ heteroduplexes are more frequent in RecA$^-$ crosses (Figure 3) than in the RecA$^+$ ones (Figure 2). This seems to tell us that the recombinants resulting from DCBs in RecA$^-$ crosses have long hybrid DNA regions, with one end at or close to the initiating DCB. In RecA$^+$ crosses, hybrid DNA is either shorter or is formed to the right of *c*I (or both). Hybrid DNA initiating some distance

from a DCB could explain the ability of a DCB to stimulate exchange on the far side of a gross heterology in RecA$^+$ but not in RecA$^-$ crosses (THALER, STAHL and STAHL 1987a).

Our observation of synthesis associated with *cos*-initiated Red-mediated exchange in RecA$^+$ cells suggests that the invading 3′ OH end generated at a DCB primes synthesis. In T4 this invasion and priming is a major mechanism for the initiation of chromosome replication—a recombination junction becomes a replication fork (for review, see MOSIG 1987). STAHL and STAHL (1986) supported the view that the small amount of DNA synthesis associated with a Red mediated exchange in a replication-blocked λ cross in RecA$^-$ cells also occurs at the site of the exchange. The possibiltiy that full-fledged replication forks can arise at Red-mediated recombination junctions is suggested by the observation (ENQUIST and SKALKA 1973) that the rate of λ DNA replication is higher in Red$^+$ than in Red$^-$ infections. Although our work fails to support that possibility, the experiments are not sensitive enough to set a low limit on its occurrence. Furthermore, since our trick for initiating exchange is somewhat contrived, our experiments do not rule out the break-copy possibility as a frequent occurrence in standard crosses nor do they bear on the possibility of such "recombination" confined to sister chromosomes (SKALKA and ENQUIST 1974).

We are grateful to BETH SAMPSON for reminding us that we had an obligation to continue previous work that was aimed at testing the possibility that λ, like T4, makes major use of double chain break-promoted recombination to initiate *ori*-independent DNA replication. Her continued interest in our efforts facilitated this project. IMRAN SIDDIQI and DAVID THALER helped us get our thoughts straight. This work, parts of which were executed in the 1970s, was supported by grants from the National Science Foundation and National Institutes of Health. F.W.S. is American Cancer Society Research Professor of Molecular Genetics.

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