Tests of the Double-Strand-Break Repair Model for Red-Mediated Recombination of Phage λ and Plasmid λdv

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ABSTRACT

The double-strand-break repair (DSBR) model was formulated to account for various aspects of yeast mitotic and meiotic recombination. In this study three features of the DSBR model are tested for Red-mediated recombination between phage λ and λdv, a plasmid that is perfectly homologous to about 10% of λ. The results support the applicability of the DSBR model to λ's Red system: (1) Creating a double-strand-break (DSB) within the region of homology shared by phage and plasmid increases their genetic interaction by about 20-fold. A DSB outside the region of shared homology has no such effect. (2) Both patches, *i.e.*, simple marker rescue, and splices, *i.e.*, co-integration of the phage and plasmid, are stimulated by a DSB in the region of shared homology. (3) Co-integrants harbor a duplication of the region of shared homology. Among co-integrants that were formed by the creation of a DSB, there is a preferential loss of whichever allele was in *cis* to a utilized cut site. The DSBR model as originally formulated involves the isomerization and cleavage of Holliday junctions to resolve the canonical intermediate. We propose as an alternative mechanism that a topoisomerase can resolve the canonical DSBR intermediate.

DOUBLE-strand-ends of DNA are local hotspots for homologous recombination in several prokaryotic (MOSIG *et al.* 1971; MICHEL, NIAUDET and EHRLICH 1982; STAHL, KOBAYASHI and STAHL 1985; SYMINGTON, MORRISON and KOLODNER 1985; STAHL 1986; THALER, STAHL and STAHL 1987) and eukaryotic (HICKS, HINNEN and FINK 1978; ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; KOLODKIN, KLAR and STAHL 1986; KUCHERLAPATI *et al.* 1984) pathways. One such pathway is the Red pathway of phage λ. The Red pathway appears to include reactions in which double-strand ends are degraded, are invasive, and prime DNA synthesis (STAHL, KOBAYASHI and STAHL 1985; STAHL and STAHL 1986). A model for such end-targeted recombination in Red has been presented (STAHL, KOBAYASHI and STAHL 1985). This model is similar in many respects to the model put forth for double-strand-break repair (DSBR) in yeast. The DSBR model of ORR-WEAVER and SZOSTAK (1983) is shown in Figure 1. Key features of this model were tested in yeast transformation experiments involving recombination between linearized plasmids and chromosomal heteroalleles.

λdv is a circularized segment of about 10% of phage λ, which replicates autonomously as a plasmid. KELLENBERGER-GUJER (1971) and BERG (1971) have shown that Red can mediate recombination between λ and λdv. λ phage growing lytically in a cell harboring λdv can "patch" (*i.e.*, rescue) a marker off the λdv or can incorporate the entire plasmid into the phage. The latter reaction may be viewed as a reciprocal "splice" (Figure 2).

In this study the restriction system for *Eco*RI is used *in vivo* to make a double-strand-cut at a defined location. In addition to λdv, the cells used in these experiments harbor the *Eco*RI restriction-modification system. The resident λdv is, of course, *Eco*RI modified, but infecting phage that are not modified will be cut by the endonuclease. Phage used in this study contain only one *Eco*RI site. *In vivo* this site becomes the location of a unique double-strand-cut. Control (uncut) experiments are performed with isogenic, but *Eco*RI modified, phage stocks.

The experiments reported in this study support three features of the DSBR model as applied to λ by λdv recombination:

1. A double-strand-cut delivered to the phage, within the region of homology shared with the plasmid, stimulates recombination between the phage and plasmid. A cut delivered outside the region of shared homology has no such effect.
2. Both patches and splices are stimulated.
3. Phage that have incorporated the plasmid contain a duplication of the region of shared homology (Figure 2B). When the phage and plasmid are distinguishable in this region, alleles in *cis* to a utilized cutsite are preferentially missing from the duplication.

MATERIALS AND METHODS

Bacterial strains, λ genetic elements and plasmids are listed in Table 1. The map positions of λ genetic elements are shown in Figure 3.

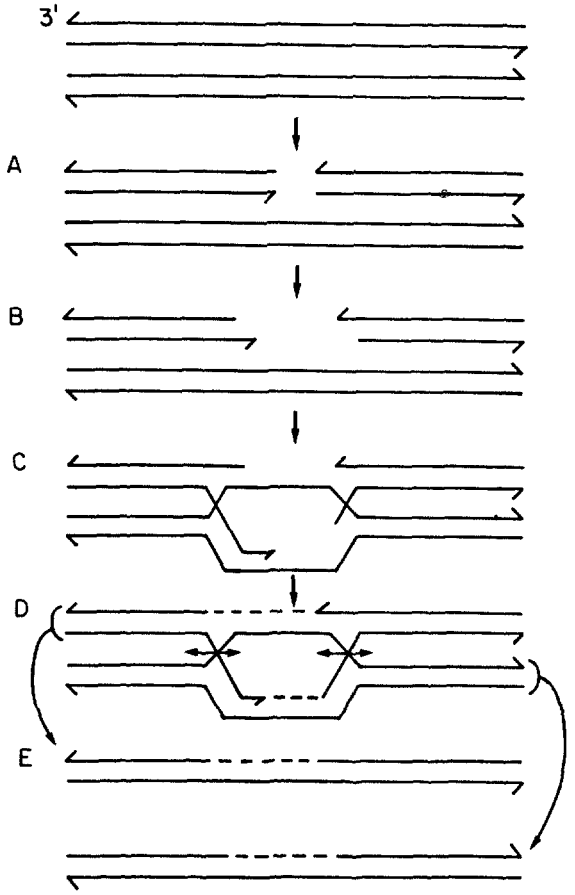

FIGURE 1.—The double-strand-break repair model of ORR-WEAVER and SZOSTAK (1983). (A) Recombination is initiated by a double-strand-cut of only one of the two interacting homologs. (B) The cut chromosome suffers a variable amount of double- and single-strand (5'-3') degradation. (C) Single strands with 3' ends are the reactive products of the cut and degradation. They invade the same uncut homolog. (D) The 3' ends each prime a round of synthesis across the gap. The result is the "canonical intermediate" structure of two HOLLIDAY junctions. The model allows the junctions to be resolved so as to give crossovers or non-crossover of flanking markers. (E) In this figure only the non-crossover (patch) mode of resolution is shown.

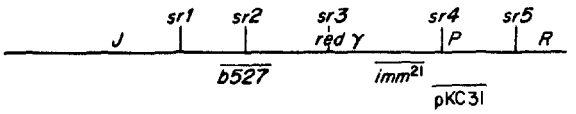

FIGURE 2.—(A) Reciprocal recombination between a circle and a rod will incorporate the circle into the rod. (B) Reciprocal recombination of pKC31 with a λ *Pam80* creates a duplication of the region of shared homology. There are three distinguishable allelic states that this duplication can have.

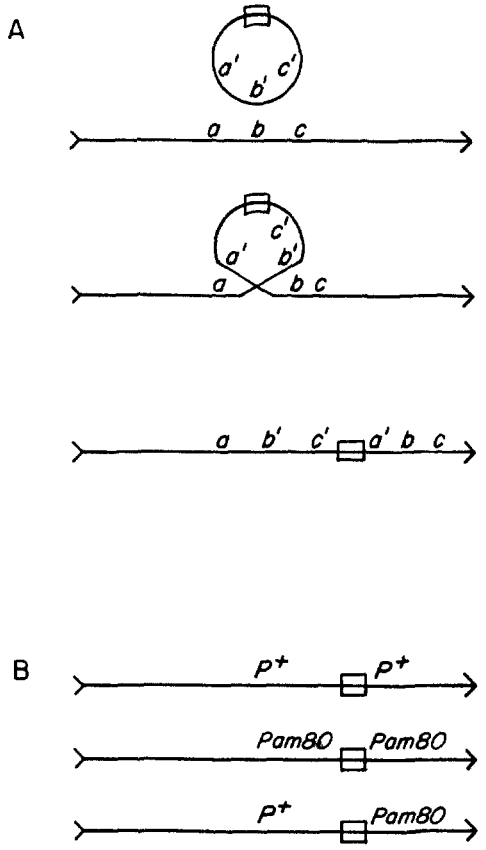

FIGURE 3.—Map of λ showing the genetic elements used in this work. The coordinates for all of the markers used, except *red3*, *210*, *imm*^{21} *cIts*, *Pam80*, *Jts15* and *Rts2*, are given in DANIELS *et al.* (1983).

λdv plasmids: pKC31 was constructed *in vitro* by Dr. R. N. RAO (E. Lilly, Indianapolis) and is identical to pRLM4 (WOLD *et al.* 1982) except that the λ segment is derived from λ *cI857 Sam7*. pKC31 is the *Bam*HI, *Hind*III fragment of λ that includes the *O* and *P* genes ligated to the *Bam*HI, *Hind*III fragment of Tn*5* that bestows kanamycin resistance. The plasmid has 4.28 kb of λ DNA and a total size of 6.15 kb.

A *Pam80* derivative of pKC31 was constructed *in vivo* using a modified version of the procedure devised by BERG (1971) for moving λdv from one host to another. pKC31 was incorporated into λ *b527 imm*^{21} *Pam80*. A population of duplication phage was isolated by cesium formate density gradient centrifugation and plated on C600 (a Su$^+$ strain, see Table 1). Phage from well separated plaques were transferred with pointed toothpicks onto lawns of Q5151 (a *groP* strain, see Table 1) and 594 (a Su$^-$ strain). A stock was grown of a duplication phage with two *Pam80* alleles (*i.e.*, a duplication phage that grew on Q5151 but not on 594) and λdv *Pam80* was "popped out" with UV (KELLENBERGER-GUJER *et al.* 1974) into bacterial strain JMB9 (*recA*$^-$ Su$^+$). Cells harboring λdv were selected and purified on kanamycin agar. Candidates were screened by gel electrophoresis of extracted DNA to identify a culture harboring a monomer λdv.

Phage strains were constructed with standard methods (ARBER *et al.* 1983); restriction sites were determined by gel electrophoresis. *Eco*RI restriction and modification functions were provided by the ampicillin-resistant, ColE1-based plasmid pMB4 (BETLACH *et al.* 1976). No problems were encountered in maintaining pKC31, or the *Pam80* derivative, and pMB4 in the same cells. The plasmids share no homology and are maintained by different selections. Kanamycin was used at 50 μg/ml and ampicillin at 100 μg/ml.

RESULTS

Experimental design: Restrictable phage DNA is cut soon after it enters the cell. In a *recBCD*$^+$ cell it is degraded quickly (SIMMON and LEDERBERG 1972).

TABLE 1

Bacterial strains, λ genetic elements, and plasmids used in this study

Designation	Relevant properties	Source, derivation or reference
A. Bacterial strains		
C600	Su^+	APPLEYARD (1954)
JMB9	C600 *recA56*	WOLD *et al.* (1982)
594	Su^-	WEIGLE (1966)
Q5151	*groP*: excludes P^+, plates *Pam80*	GEORGOPOULOS and HERSKOWITZ (1971)
FS1757	Streptomycin resistant Q5151	This work. Streptomycin resistance transduced from 594
B. λ Genetic elements		
Jts15	Temperature-sensitive allele of *J*	BROWN and ARBER (1964)
b527	Deletion of 8.3% of λ left of attachment site; covers *Eco*RI site 2	PARKINSON (1971)
imm^{21}	Substitution of phage 21 immunity into λ	Phage 21: JACOB and WOLLMAN (1961); substitution endpoints: DANIELS *et al.* (1983)
Pam80	Suppressor-sensitive allele of *P*, can be positively selected on Q5151	CAMPBELL (1961)
Rts2	Temperature-sensitive allele of *R*	BROWN and ARBER (1964)
*Eco*RI $sr1^0$, $sr3^0$, $sr4^0$, $sr5^0$	Uncleavable *Eco*RI sites	RAMBACH and TIOLLAS (1974); MURRAY and MURRAY (1979)
χ^+A	Chi sequence left of the *b* region	See STAHL *et al.* (1982)
χ^+D	Chi sequence right of *P*	See STAHL *et al.* (1982)
imm^{21} *cIts*	Temperature-sensitive allele of *c*I of imm^{21}	Laboratory collection
C. Plasmids		
pMB4	Ampicillin-resistant *Eco*RI restriction and modification plasmid	BETLACH *et al.* (1978)
pKC31	Kanamycin-resistant λdv	R. N. RAO
λdv *Pam80*	*Pam80* derivative of pKC31	This work

ExoV, the product of the *recB, C* and *D* genes (AMUNDSEN *et al.* 1986; BIEK and COHEN 1986), is also inhibitory to Red recombination (STAHL and STAHL 1974) and may be important for the maintenance of λdv (KELLENBERGER-GUJER *et al.* 1974). We use a helper phage in order to have active Red and Gam functions in the cell before the restrictable phage enter. The helper phage is *Eco*RI-modified and has the same alleles, in the region of λdv homology, as does the experimental phage. Unlike the experimental phage, the helper is *Jts Rts* and has a temperature-sensitive allele in *c*I. All phage used in this study are imm^{21}. Phage that are imm^{21} grow without inhibition in hosts harboring λdv (MATSUBARA and KAISER 1968). Bacterial cultures are infected with the helper phage 10 min before adsorption of the experimental phage is begun. Assay plates of the lysates were incubated at temperatures restrictive for the helper phage for the experiments reported in Tables 2 through 6. The density gradients shown in Figure 4 were plated at both high and low (*i.e.*, restrictive and permissive) temperatures for the helper phage. The crosses were conducted at 37°.

Cutting inside the region of shared homology increases the proportion of the phage yield that has picked up a marker from the λdv: The data in Table 2 are from experiments with the *Pam80* allele on the input experimental phage and on the helper. The

TABLE 2

Effect of cutting of *Eco*RI site 4 on phage (*Pam80*) by λdv (P^+) recombination

Experiment	Percent of total yield that plates on 594		Hotspot = cut/uncut
	Cut	Uncut	
1	9.1	0.48	19
2	2.6	0.08	32
3	6.6	0.34	19
Average ± SD	6.1 ± 3.3	0.30 ± 0.20	23 ± 7.5

JMB9 [pKC31] [pMB4] cultures at 2×10^8 cells/ml were infected with *Eco*RI-modified λ*Jts15* imm^{21} *Pam80 Rts2* at a multiplicity of infection (m.o.i.) of 5. After 10 min shaking λ$sr1^0$ *b527* $sr3^0$ imm^{21} *Pam80* $sr5^0$ was added, also at an m.o.i. of 5. After an additional 10 min the cultures were diluted 50-fold, and a sample was removed and chloroformed to measure unadsorbed phage. After an additional hour post dilution at 37° the cross was chloroformed. Yield was titered on 594 and C600, both at 42°. For uncut crosses the λ$sr1^0$ *b527* $sr3^0$ imm^{21} *Pam80* $sr5^0$ stock was *Eco*RI modified.

experimental phage has only the number 4 *Eco*RI site. At 39.168 kb, this site is near the border of the *O* and *P* genes. With respect to the region of homology shared by phage and plasmid, the site is 1.709 kb from the left end and 2.564 kb from the right end.

If the experimental phage is restricted *in vivo* at the number 4 site, the proportion of P^+ phage in the total phage yield is about 20-fold higher than if the experimental phage entered the cross *Eco*RI-modified. The

TABLE 3

Effect of cutting at *Eco*RI site 4 on phage (P^+) by λdv(*Pam80*) recombination

Experiment	Percent of total yield that plates on Q5151		Hotspot = cut/uncut
	Cut	Uncut	
1	3.0	0.19	16
2	2.7	0.15	18
3	3.3	0.17	19
Average ± SD	3.0 ± 0.3	0.17 ± 0.02	18 ± 1.5

JMB9 [pKC31/*Pam80*] [pMB4] cultures at 2×10^8 were infected with *Eco*RI-modified λ*Jts15 imm*^{21} *Rts2* at an m.o.i. of 5. After 10 min shaking, the cultures were superinfected with *sr*1^0 *b527 sr*3^0 *imm*^{21} *sr*5^0 phage and shaken for an additional 10 min. Cultures were then diluted 50-fold, and unadsorbed phage samples were removed and chloroformed. The rest of the cross was shaken for another hour at 37°, then chloroformed. The cross was titered on Q5151 at 37° and C600 at 42°.

same type of experiment was performed with the starting positions of the input alleles reversed, *i.e.*, with *Pam80* on the λdv and P^+ on the experimental and helper phage. Results of these reversed marker experiments are shown in Table 3. In this set of experiments cutting on the experimental phage increased the proportion of the phage yield that is *Pam80* by an average of 18-fold. These experiments demonstrate that cutting within the region of shared homology increases the recombinogenic interaction of phage and plasmid.

Cutting outside the region of shared homology does not increase marker pickup from the λdv: The number 5 *Eco*RI site is at 44.972 kb. This is 3.240 kb to the right of the region of shared homology. A phage was constructed, identical in every respect to the *Pam80* experimental phage, except that this new phage was *Eco*RI *sr*4^0 (*i.e.*, not cuttable at the number 4 *Eco*RI site) and *Eco*RI *sr*5$^+$ (*i.e.*, wild type at the *Eco*RI site 5). Experiments were conducted with cuttable and with *Eco*RI-modified *sr*4$^+$ *sr*5^0 and *sr*4^0 *sr*5$^+$ stocks in parallel aliquots of the same bacterial culture. The results of these experiments are shown in Table 4. *Eco*RI-modified stocks of *sr*4$^+$ *sr*5^0 or *sr*4^0 *sr*5$^+$ phage recombine equally well with λdv. However when the experimental input phage are not *Eco*RI modified, and hence are cuttable, *sr*4$^+$ *sr*5^0 and *sr*4^0 *sr*5$^+$ yield different results. As in the previous set of experiments, cutting *in vivo* at the *sr*4$^+$ site increases the proportion of the yield that has become P^+. Cutting *in vivo* at the *sr*5$^+$ site does not change the proportion of the phage yield that has picked up a marker from the λdv.

Both patches and splices are stimulated by cutting within the region of shared homology: Equilibrium density gradient centrifugation can resolve phage that have incorporated a plasmid from those that have not. Such an analysis with both cuttable and modified *sr*4$^+$ experimental phages is shown in Figure 4. Several features of these gradients are worth noting. The effect of cutting can be expressed as one number, the "hotspot" value:

$$\frac{\frac{P^+}{\text{total}}\ \text{cut}}{\frac{P^+}{\text{total}}\ \text{uncut}}.$$

For this experiment the hotspot value is about 30, among phage that are not temperature sensitive.

The lightest peak in these gradients is of the genotype *b527 imm*^{21}. The heaviest peak is composed of *b527 imm*^{21} phage that have incorporated the 6.1-kb plasmid pKC31. The gradients were assayed on Su$^+$ and Su$^-$ indicators at 32° and at 42°. A temperature of 32° is permissive for the *Jts* and *Rts* alleles on the helper phage. *Pam80* will not plate on Su$^-$ at any temperature. P^+ will plate on Su$^-$ at both 32° and 42°.

Among phage that plate on Su$^-$ at 42°, from the cross in which recombination was stimulated by restriction, the lightest peak is 1.5 times the height of the heaviest peak (Figure 4A; compare fractions 16 and 27 titered on Su$^-$). In the parallel experiment in which recombination was not stimulated by restriction, the corresponding value is 1.1 (compare fractions 11 and 20 titered on Su$^-$).

At 32°, a new peak appears. The new peak is especially prominent on Su$^+$ and is intermediate in density between the lightest and heaviest peaks. This intermediate density is appropriate for the helper phage, which is *imm*^{21} but does not possess the deletion *b527*. The helper phage is too large to incorporate a λdv plasmid and remain within the size limitation for packaging (FEISS and BECKER 1983).

In the experiment in which recombination was stimulated by restriction (Figure 4A) the titer at 32° on Su$^+$ at the lowest density (fraction 27; △) is higher than the same fraction titered at 42° on Su$^+$ (▲). In the experiment in which recombination was not stimulated by restriction (Figure 4B), the titer of the lightest peak on Su$^+$ is nearly identical at 32° and at 42°. Restriction of the ts$^+$ experimental phage (*i.e.*, the phage that is either restricted or not in Figure 4, A and B, respectively) probably stimulates splicing between the experimental phage DNA and the helper phage DNA (*i.e.*, the phage that is not restricted in either cross and is of the genotype *Jts imm*^{21} *Pam80 Rts*). The result of restriction-stimulated recombination between the experimental and the helper phage is the creation of the genotypes *Jts b527 imm*^{21} *Pam80* and *b527 imm*^{21} *Pam80 Rts*. Restriction-stimulated phage by phage splicing has been demonstrated in

TABLE 4

Cutting outside the region of shared homology has no stimulating effect on recombination

	P^+/Total (%)					
	$sr4^+$ $sr5^0$			$sr4^0$ $sr5^+$		
Experiment	Cut	Uncut	Hotspot	Cut	Uncut	Hotspot
1	0.62	0.066	9.4	0.061	0.066	0.92
2	11	0.59	19	0.94	0.74	1.3
3	4.5	0.49	9.2	0.52	0.49	1.1
Average ± SD	5.4 ± 5.2	0.38 ± 0.3	13 ± 5.6	0.51 ± 0.44	0.43 ± 0.34	1.1 ± 0.19

Crosses were conducted as described in Table 2. All phage are $sr1^0$ *b527* $sr3^0$ imm^{21} *Pam80*.

TABLE 5

Cutting inside the region of shared homology increases the proportion of patches

	Cut		Uncut	
Experiment	Patch %	Splice %	Patch %	Splice %
1	60	40	42	58
2	65	35	39	61
3	59	41	48	52
Average ± SD	61 ± 3.2	39 ± 3.2	43 ± 4.6	57 ± 4.6

JMB9 [pKC31] [pMB4] cultures at 2×10^8 cells/ml were infected and treated as described in the legend to Table 2. Well separated plaques on 594 were stabbed to kanamycin and non-kanamycin agar and incubated 24 hr at 37°. Those stabs that gave rise to kanamycin-resistant colonies were scored as splices.

TABLE 6

Effect of cutting on the allele state of duplication phage

	Duplication phage that are (%):			No. of plaques
Allele state	P^+/P^+	*Pam80/Pam80*	P^+/*Pam80*	tested
A. Input phage and helper *Pam80*, λdv P^+				
Cut	53	4	43	480
Uncut	20	19	61	464
B. Input phage and helper P^+ λdv *Pam80*				
Cut	11	43	46	400
Uncut	27	14	59	390

Crosses were conducted as described in the legend for Table 2 (for A) and Table 3 (for B). Cross lysates were centrifuged to equilibrium in cesium formate. From the heavy peak, phage were plated on C600, and well separated plaques were scored as described in the text.

other work (THALER, STAHL and STAHL 1987).

Further evidence of restriction-stimulated recombination between the experimental phage and λdv can be seen in the gradients as titered at 32° on Su^- (○ in Figure 4). The temperature-sensitive helper phage is *Eco*RI modified in both crosses and hence never restricted. If the experimental phage is restricted (Figure 4A), the 32° P^+ titer at the density of the helper phage (fraction 22 ○) is approximately one-third the titer of P^+ phage at the density of experimental phage that have obtained the P^+ marker and have, or have not, incorporated the λdv (fractions 16 and 27 ●, respectively). The corresponding cross without restriction (Figure 4B) yields more helper phage that have become P^+ (fraction 22; ○ in Figure 4) than experimental phage that have become P^+ (●) whether or not the experimental phage have incorporated the λdv (fractions 11 and 20, respectively).

During the course of this work we found that phage that have spliced-in the plasmid pKC31 confer kanamycin resistance to lysogens. All of 100 turbid plaques on Su^- from the 42° "splice" peak in the cut cross (fraction 16 in Figure 4A) conferred kanamycin resistance by this criterion. None of 100 plaques from the "patch" peak conferred resistance. Kanamycin resistance provides an alternative way to identify phage that have spliced-in the λdv.

Three experiments were conducted without centrifugation using kanamycin resistance to score splices. The phage yield was plated at 42° on 594. Well separated plaques (all plaques were turbid) on 594 were stabbed with a sterile toothpick and transferred onto kanamycin and non-kanamycin agar. All plaques contained bacteria that grew on non-kanamycin agar. Those plaques containing bacteria that also gave growth on kanamycin agar were scored as splices. The results of the analysis are shown in Table 5. As in the gradients of Figure 4, it can be seen that cutting elevates the proportion of patches relative to splices among phage that have picked up the P^+ marker from the λdv. We do not offer any models for the observation that cutting stimulates patches more than splices.

Alleles in *cis* to a utilized cutsite are preferentially lost: Phage that have incorporated a λdv contain a duplication of the region of shared homology. Experimental phage and plasmid in these experiments differ in their allele at *P*. Duplication phage have three possible states with respect to their alleles at *P*: P^+/P^+, *Pam80/Pam80*, or P^+/*Pam80*. The direct repeat in duplication phage may contain two copies of P^+ or *Pam80* information, or phage may be duplication heterogenotes which contain one copy of P^+ and 1 copy of *Pam80* information. The alternative forms of duplication phage are illustrated in Figure 2.

BERG (1971) scored heterogenotes by plating dupli-

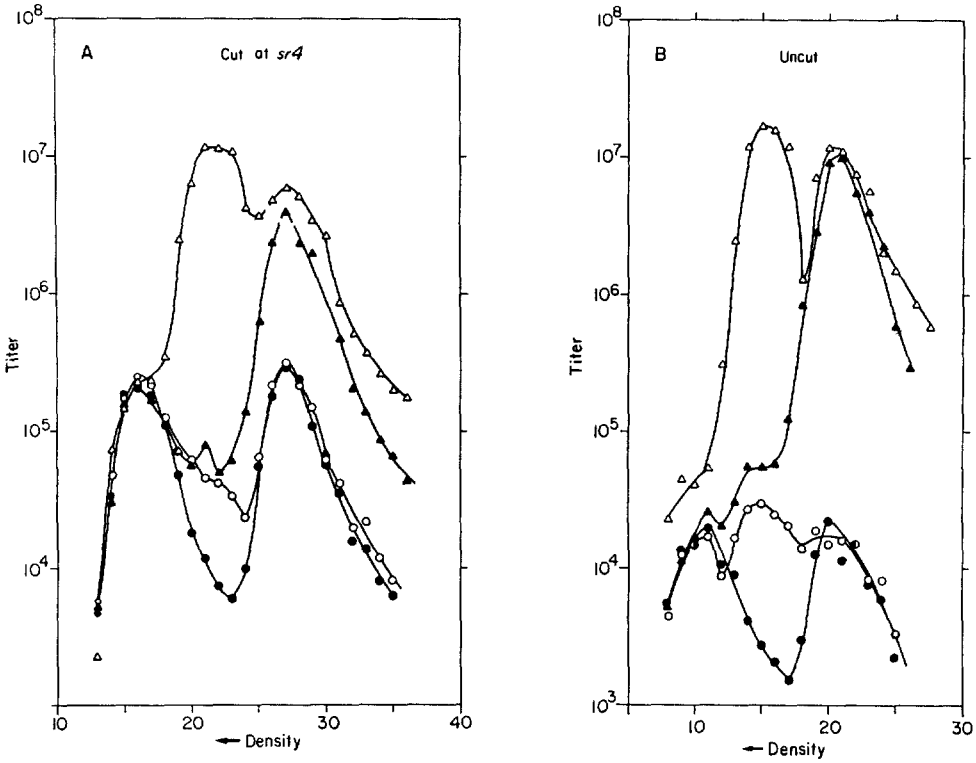

FIGURE 4. Density gradient analysis of the cross *sr1*^{0} *b527 sr3*^{0} *imm*^{21} *Pam80 sr5*^{0} in JMB9[pKC31]. The helper phage is *Eco*RI-modified *Jts15 imm*^{21} *clts Pam80 Rts2*. In panel A the *sr1*^{0} *b527 sr3*^{0} *imm*^{21} *Pam80 sr5*^{0} phage is restricted *in vivo* at the *sr4*$^+$ site which is within the region of homology shared by the phage and λdv. In panel B all phage stocks enter the cross *Eco*RI modified. The gradients were titered on: 594 at 42° (●), C600 at 42° (▲), 594 at 32° (○), and C600 at 32° (△).

cation phage, isolated on the basis of density, on Su$^-$, stabbing plaques and transferring a sample onto *groP*. We worried that the *Pam80* allele might be lost during plaque growth on the Su$^-$ host. In this study we plated the duplication phage, similarly isolated on the basis of density, on a Su$^+$ lawn. Plaques from the Su$^+$ lawn were stabbed with a sterile toothpick that was then stabbed sequentially onto kanamycin agar, a *groP* lawn, a Su$^-$ lawn, and a Su$^+$ lawn. The first stab was used to confirm the presence of the kanamycin resistance element and hence the plasmid in the phage. The second stab, onto *groP*, was a positive selection for the presence of a *Pam80* allele. The third stab, onto Su$^-$, was a positive selection for the presence of a *P*$^+$ allele. The fourth stab, onto Su$^+$, confirmed the presence of phage on the toothpick. The experiment was performed with both marker configurations, *i.e.*, with *P*$^+$ or *Pam80* on the λdv and the other marker on both phage. The results of these analyses are shown in Table 6. In either marker configuration the allele on the experimental phage was lost preferentially when the phage was cut.

A reconstruction experiment indicates that duplication heterogenotes were not converted to duplication homogenotes during growth in the initial Su$^+$ plaques. Five plaques that were duplication heterogenotes were picked, each into 1 ml of buffer, diluted appropriately and replated on Su$^+$ for well separated plaques. They were then retested by testing 40 plaques from each through the series just described. All plaques that retained the plasmid, *i.e.*, contained kanamycin resistant lysogens, were still heterogenote, *i.e.*, they still gave plaques when stabbed onto both *groP* and Su$^-$ lawns.

DISCUSSION

Points of agreement with DSBR model: Three results of this study support the view that the DSBR model as proposed for yeast (ORR-WEAVER and SZOSTAK 1983, SZOSTAK *et al.* 1983) provides an economical account of Red-mediated λ by λdv recombination:

1. Creating a double-strand-end inside the region of shared homology increases phage by plasmid recombination by about 20-fold (Tables 2 and 4, Figure 4).

2. Cutting increases both patches and splices (Figure 4). In the next section we present alternative models for resolution in which neither splices nor patches are made via the resolution of cross-strand junctions according to the rules of HOLLIDAY (1964).

3. Among recombinants that have spliced-in the plasmid there is a loss of whichever allele was in *cis* to a utilized cut site (Table 6). This fulfills a prediction of the DSBR model in which a recombinator sacrifices itself in the act of stimulating recombination. According to the DSBR model the loss of alleles in *cis* to a utilized cut site is a consequence of exonuclease action starting at the cut site. Alternatively one could account for the loss of alleles in *cis* to a utilized cut site by supposing an asymmetry of mismatch correction

which is biased against the chromosome that initiated recombination. A definitive demonstration of conversion by gap filling has been achieved in the largely homologous RecE pathway (I. KOBAYASHI, personal communication). It is worth noting that in contrast to the results presented here for DSBR recombinators, Chi, a recombinator in the RecBCD pathway, does not sacrifice itself in the act of stimulating recombination (STAHL, LIEB and STAHL 1984). Two gaps in our knowledge about Red make the fit of Red to the canonical DSBR model less than certain: (a) it is not known if simple reciprocal recombination is the basis of λ by λdv interaction, and (b) there is no evidence for a HOLLIDAY junction resolving activity being involved in Red recombination.

With respect to the first point, proof of single event reciprocal interaction has been elusive in phage systems (SARTHY and MESELSON 1976; STAHL 1979). Neither has such proof been obtained for yeast transformation, the system for which the DSBR model was designed. The assumption of a single reciprocal interaction being the basis of phage by plasmid, as well as gapped DNA by chromosome, recombination is justified by simplicity.

The DSBR model supposes that only the strand that ends 3′ can act to prime repair synthesis. The 3′ tips of the new strands are thought to pass each other as they are being synthesized in opposite directions. The result is the complete synthesis of both strands, *i.e.*, double-strand-gap filling. An alternative worth consideration is that an invading duplex could initiate a full replication fork, *i.e.*, synthesis on both the leading and the lagging strand. Initiation of full replication forks primed from the tips of invading duplexes is the major mode of late T4 DNA replication (LUDER and MOSIG 1982, DANNENBERG and MOSIG 1981, 1983). STAHL and STAHL (1986) have demonstrated repair synthesis initiated from a Red-mediated strand invasion. The experiments of STAHL and STAHL (1986) were conducted at high temperature in a *dnaBts* strain. *dnaB* may not be required for repair synthesis on one strand, but may be required for a full replication fork. The experiments reported in this paper were conducted under conditions permissive for replication. If under conditions permissive for replication the invading duplex can prime a full replication fork, then it is easier to entertain models that involve multiple sequential nonreciprocal events.

An alternative mode of resolving: The DSBR model as originally proposed involves the formation of an intermediate consisting of a region of gap filling bounded on each side by a HOLLIDAY junction. Resolution of the intermediate is proposed to proceed via the cleavage of both HOLLIDAY junctions. At least one of the two HOLLIDAY junctions is postulated to be free to isomerize. Thus half of the products of resolving the intermediate are predicted to be recombinant for markers flanking the region synthesized during gap repair and the other half are predicted to maintain parental flanking markers. Resolving this canonical intermediate by cleavage of the two HOLLIDAY junctions is an economical model that dovetails with three key observations garnered from studies of yeast recombination:

1. In transformants of yeast with linearized DNA, among those transformants that have experienced gap-repair approximately one-half are integrated into the chromosome from which they were repaired and the other half are recovered as free plasmids (ORR-WEAVER and SZOSTAK 1983).

2. In yeast meiosis, among tetrads that have converted at a given locus approximately one-half are recombinant for flanking markers, *i.e.*, the tetrads are tetratype for markers flanking the region of gene conversion (SZOSTAK *et al.* 1983).

3. Gene conversion in meiosis can be induced by a double-strand-cut, and among the induced conversion asci approximately one-half are tetratype for markers flanking the region of conversion (KOLODKIN, KLAR and STAHL 1986).

We have noticed that there is another way to resolve the canonical intermediate, a way that does not involve cutting of any HOLLIDAY junctions. The intermediate as drawn resembles DNA structures that can be formed and resolved by some of the known topoisomerase activities in both *E. coli* and in yeast (DRLICA 1984; DINARDO, VOELKEL and STERNGLANZ 1984). LEACH and STAHL (1983) suggested that gp*recBC* and gp*sbcB* are involved in HOLLIDAY junction resolution because "knock-out" of these genes allows phage carrying perfect palindromes to plate. Perfect palindromes may form cruciforms *in vivo*, structures of local stereochemistry equivalent to HOLLIDAY junctions. Red function, however, was not harmful to palindromes in their study. The implication is that Red does not encode HOLLIDAY junction cleaving activity. On the other hand, there may be a HOLLIDAY-junction cleaving activity in the Red pathway, but one that can distinguish between HOLLIDAY junctions arising by recombination and four-way junctions at the base of cruciforms.

A model has been advanced in which nonreciprocal splicing between two λs does not require HOLLIDAY junction resolving activity (STAHL, KOBAYASHI and STAHL 1985; STAHL and STAHL 1985). We therefore consider other models for phage by plasmid Red-mediated recombination. Two models are illustrated in Figure 5. The features of the topoisomerase model, illustrated in Figure 5, A and B, for λ by λdv recombination are:

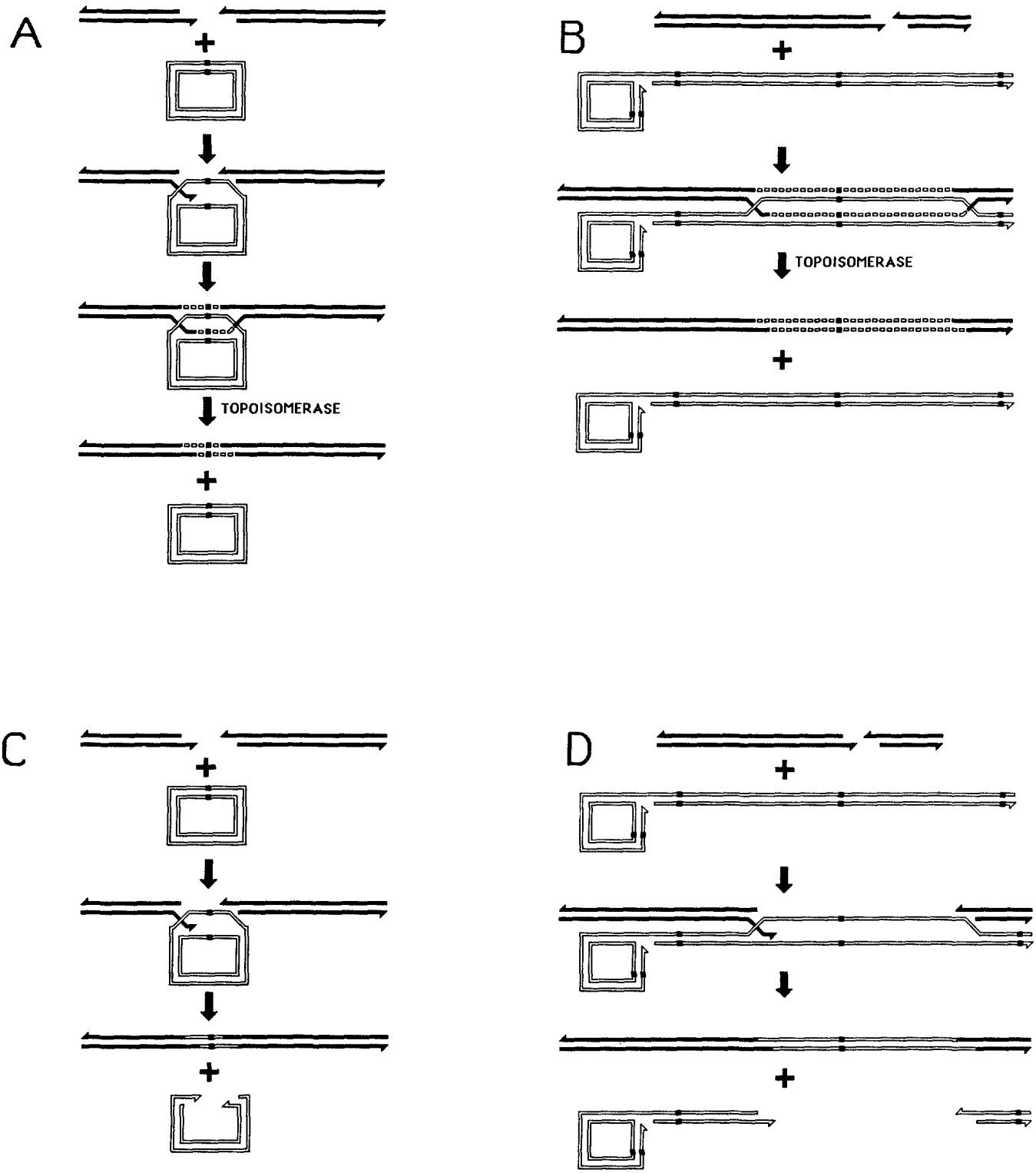

FIGURE 5.—(A) An alternative way to resolve the canonical DSBR intermediate. In this model we suppose that the intermediate is resolved simply by lifting upwards on the two ends of the originally cut parent. In reality the DNA is presumed to be intertwined so a winding/unwinding activity is required. A consequence of the pull-out mode of resolution is that the two new strands synthesized during gap repair are conservatively inherited, *i.e.*, both present in only one of the two products of recombination. In contrast the ORR-WEAVER and SZOSTAK (1983) mode of resolution distributes the newly synthesized strands semiconservatively (see Figure 1). (B) The mechanism described above should be able to acquire only patches in the system of λ by λdv. In order to account for splices, *i.e.*, incorporation of the plasmid into the phage, we suppose that: (1) the λdv rolls out into a linear concatamer, (2) the two ends of a cut phage invade consecutive direct repeats and prime synthesis across a monomer unit, and, (3) the intermediate is resolved as in (A). (C) A gap-transfer mechanism for stimulating recombination with a double-strand-cut. Each invading end is supposed to catalyze a break-join reaction with the originally uncut homolog. If both ends invade the same monomer unit the result is a patch, *i.e.*, a pickup of a genetic marker from the originally uncut homolog. (D) A gap-transfer mechanism for incorporating the plasmid into the phage. Assume that the plasmid has formed a concatamer and that the ends invade adjacent direct repeats. Otherwise the reaction is identical to (C).

1. The canonical DSBR intermediate is formed.
2. Resolution of the canonical intermediate is not via cleavage of HOLLIDAY junctions as proposed by ORR-WEAVER and SZOSTAK (1983) and SZOSTAK *et al.* (1983).
3. Resolution is via the winding/unwinding activity of a topoisomerase, such as gyrase in *E. coli* or topoisomerase type II in yeast.

The mechanism described is sufficient to get patches off a plasmid, but not to get splices, *i.e.*, incorporation of the plasmid into the phage. In order to account for splices through this mechanism we make an addition:

4. Under the conditions of the cross, (*gam*$^+$), λdv goes into a rolling circle mode of replication. This has been directly confirmed for pKC31, the λdv used in this study (COHEN and CLARK 1986).

If the two ends of a cut λ can invade successive direct repeats of a linear concatamer and join via gap-repair, then the same resolution mode proposed in 3) above would give a "splice." This is illustrated in Figure 5B.

Some predictions of the pull-out mode of resolution that distinguish it from a HOLLIDAY cleavage mode of resolution are:

1. The pull-out model predicts that both newly synthesized strands will become part of the recombinant product that was originally cut. The HOLLIDAY cleavage model predicts that each recombinant double helix will have one old and one new (*i.e.*, synthesized during gap repair) strand.
2. A specific role for a topoisomerase activity in resolution is postulated for the pull-out model.
3. "Splices" in the pull-out model are dependent on concatamer formation.

A related model (Figure 5, C and D) supposes that ends invade but that the invading ends do not prime DNA synthesis. Instead, suppose a break-join reaction at each invading end. The result is the transfer of a DNA segment from the originally uncut homolog into the gap that initiated the reaction. We will call this mechanism "gap-transfer" because the reaction results in the transfer of a gap from the originally cut homolog onto the homolog that was originally intact. The "gap-transfer" model does not invoke topoisomerase for resolution but does invoke rolling circle concatamers as essential to the formation of splices.

In considering the models presented in this section it is important to note that both reactions, *i.e.*, break-join and synthesis primed from an invading end, have been documented in Red-mediated recombination at an invading end (STAHL, KOBAYASHI and STAHL 1985; STAHL and STAHL 1986). The experiments presented in this study do not allow a distinction between either of the conservative segregation models or between conservative segregation and the semiconservative segregation predicted by the ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) DSBR model. It is clear that each of the three models makes different predictions regarding the relationship of material and information transfer. In sum: (a) The DSBR model (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) predicts synthesis of both strands across the gap and semiconservative segregation of the new DNA generated by gap repair. The DSBR model allows for bidirectional transfer of material and information in the heteroduplex regions which bound the region of gap repair. (b) The topoisomerase-pull-out model predicts synthesis across the gap and the subsequent formation of an intermediate identical to that predicted by the DSBR model. This intermediate is predicted to be resolved conservatively with all newly synthesized DNA ending up filling the gap that originally provoked the reaction. The uncut homolog bears no scar, physical or genetic, of having been involved in a reaction.

3. The gap-transfer model, illustrated in Figure 5, C and D, predicts that each invading end provokes a break-join reaction. The net result is that the DNA corresponding to the gap is "stolen" from the originally uncut homolog. After one cycle of this reaction the originally uncut homolog is in a state similar to the gapped chromosome that initiated the reaction. No DNA synthesis is invoked in the gap-transfer model.

A contrast with the results of other plasmid by phage recombination studies: SHEN and HUANG (1986) were unable to get the RecE pathway to splice a plasmid into a λ with which it shared homology. This surprising result has been confirmed by KING and RICHARDSON (1986). Both papers argue that Red and RecE are inherently nonreciprocal and thus not capable of mediating the incorporation of a plasmid into a phage. However, as demonstrated in this work and previously by others (BERG 1971; KELLENBERGER-GUJER 1971), Red is capable of mediating the incorporation of a λdv plasmid into λ. We suggest an alternative explanation; perhaps the crucial difference is that the plasmid used in the work of SHEN and HUANG (1986) and KING and RICHARDSON (1986) is ColE1 based. COHEN and CLARK (1986) have shown that a ColE1 based plasmid is much less prone to go into a rolling circle mode of replication than is a λdv plasmid. We predict that RecE would be found competent to mediate patch recombination between λ and a ColE1 based plasmid.

A second explanation offered by SHEN and HUANG (1986) as well as KING and RICHARDSON (1986) is that the RecBCD pathway is able to promote crossing over between smaller stretches of homology than is the RecE, and by inference the Red, pathway. The largest

homology shared by phage and plasmid in the study of SHEN and HUANG (1986) is 405 bp, whereas in this study the region of shared homology is 4.28 kb.

HENRY HUANG (St. Louis) noted a third possibly significant difference: the λ origin of replication (*ori*) is included in the region of homology shared by phage λ and plasmid λdv. λ's *ori* may experience a site-specific nick (ENQUIST and SKALKA 1973) that could be recombinogenic. It must also be noted that the experiments reported in this paper utilize the Red pathway, whereas those of SHEN and HUANG (1986) use the RecE pathway. The Red and RecE pathways are homologous (KAISER and MURRAY 1979), but they are not equivalent in all respects (SMITH 1983, Table 1). In sum, the differences between our results and those of SHEN and HUANG (1986) may be due to differences in: (a) length of homology shared by the phage and plasmid, (b) site-specific nicks that occur at the λ *ori*, (c) the Red *vs.* the RecE pathway and (d) the tendency of λdv to roll its circle more than does ColE1, and perhaps differently from that plasmid. Our control experiments conducted without cutting agree with previous work on Red-mediated λ by λdv recombination (BERG 1971; KELLENBERGER-GUJER 1971).

The data presented in this paper are easily interpretable in the context of the DSBR model as articulated by ORR-WEAVER and SZOSTAK (1983). In the DSBR model, both ends created by a double-strand-cut invade the same uncut homolog and both invading ends prime repair synthesis. A symmetric intermediate is formed that consists of a region of gap repair bordered on each side by a HOLLIDAY junction. In a previous study (THALER, STAHL and STAHL 1987) we have presented evidence that a symmetric intermediate is not often formed during cut-stimulated splicing between the two λ phages. A symmetric intermediate, *i.e.*, one in which both ends created by a cut invade the same uncut homolog, is not a requirement for λ by λ splicing. It may be that a symmetric intermediate is a requirement for the recovery of viable products of λ by λdv recombination whether these products are patches or splices. Alternatively, the results of our study may be the consequence of multiple exchanges each of which is asymmetric.

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