Heteroduplex Chain Polarity in Recombination of Phage λ by the Red, RecBCD, RecBC(D⁻) and RecF Pathways

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ABSTRACT

We have examined the chain polarity of heteroduplex DNA in unreplicated, bacteriophage λ splice recombinants when recombination was by the RecBCD, RecBC(D⁻), or RecF pathway of *Escherichia coli* or the Red pathway of λ. For each of these pathways, recombination is activated by the cutting of *cos* that accompanies chromosome packaging, and is effected by recombination enzymes acting at the right end created by that cutting. For exchanges occurring near *cos*, one parent makes a lesser physical and genetic contribution than does the other. For each pathway, when the phage carried standard *cos*, this minority contribution was predominantly on the *r* chain, ending 5′ at the right end of λ. When standard cos was replaced by a cloned inverted *cos located centrally on the standard* λ genetic map, minority contribution was predominantly on the *l* chain. In each case, the polarity of the overlap was usually that formed by 3′ overhangs of parental information and material. These results are discussed in the context of current models of recombination for the different pathways.

PHAGE λ can undergo generalized recombination in its *Escherichia coli* host by any of four catalyzed pathways: *E. coli*'s RecBCD, RecF and RecE pathways, and λ's Red pathway (for recent reviews see CLARK and LOW 1988, MAHAJAN 1988, and SMITH 1987). The first three were originally defined for conjugative recombination; with some modifications those definitions are applicable to λ recombination (THALER *et al.* 1989; see below). These pathways differ in their response to a DNA double-chain break (DCB) (reviewed in STAHL 1986 and THALER and STAHL 1988). On this basis the Red, RecE and RecF pathways form a class that we shall here call DCB pathways (STAHL, KOBAYASHI and STAHL 1985; THALER, STAHL and STAHL 1987a; KOBAYASHI and TAKAHASHI 1988; THALER *et al.* 1989; TAKAHASHI and KOBAYASHI 1990). They share the ability to utilize duplex ends as substrates for recombination, with the exchange event occurring at or near the end itself. This commonality is reflected in several features of the exchange event for λ recombination. These features differ from those of the RecBCD pathway, in which the RecBCD enzyme can carry out recombination far from the DCB, after encountering a properly oriented χ sequence (STAHL 1986).

cos (cohesive end site) is the site of a DCB made by the packaging enzyme terminase (FEISS and BECKER 1983). Among progeny particles carrying unreplicated chromosomes, an interval close to *cos* undergoes proportionately more recombination by DCB pathways than does a distal interval. Recombination is also stimulated by a DCB delivered by a type II restriction enzyme (THALER, STAHL and STAHL 1987a,b; THALER *et al.*1989; STAHL *et al.* 1990a). In each case there is a stimulation of the proportion and amount of recombination in an interval containing the cut site.

Among replicated chromosomes, recombination is more evenly distributed along the chromosome (STAHL *et al.* 1972b; GILLEN and CLARK 1974; THALER *et al.* 1989). A model that seeks to explain this by a break-copy mechanism proposes that recombination events are initiated by breakage throughout the length of the λ chromosome. When replication is limited, only those events that initiate near *cos* can complete the DNA synthesis necessary to form a mature recombinant (STAHL, KOBAYASHI and STAHL 1982). For the Red system acting in *recA* cells, this break-copy scheme was ruled out as the only recombination mechanism by the demonstration that *cos* can promote recombination even when that *cos* cannot be used for packaging (STAHL, KOBAYASHI and STAHL 1985). The *cos* that stimulates recombination fails to appear in the recombinant.

An alternative model proposes that double chain ends are the only initiators of recombination. When replication is blocked, such ends occur only at *cos*. Under conditions permissive for replication, the tips of rolling circles are randomly distributed across the chromosome and serve to initiate recombination in intervals far from *cos* (WILKINS and MISTRY 1974; STAHL, KOBAYASHI and STAHL 1985). Recombination in this view proceeds by break-join. Evidence in support of this model was gained by the demonstration

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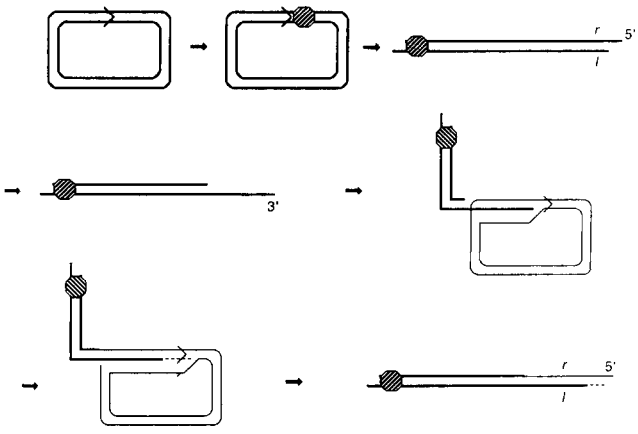


FIGURE 1.—A double-chain break model for *cos*-initiated λ recombination (from STAHL, KOBAYASHI, and STAHL 1985). Terminase binds to and linearizes the heavy-line chromosome at *cos*. The free end undergoes 5′ → 3′ exonucleolytic digestion. The 3′ single chain end invades an uncut homolog (thin lines). Appropriate nicks and ligations and DNA synthesis (dashed line) lead to the formation of a recombinant in which the uncut parent makes a minority material contribution in the heteroduplex region on the 5′ ending *r* chain.

that the phage Mu γ protein, which binds to double-chain ends, inhibits Red recombination in *recA* cells permissive for DNA replication (THALER, STAHL and STAHL 1987c). Further, STAHL *et al.* (1990a) introduced a double chain break far from *cos* in one parent. Many of the induced recombinants were formed with little or no DNA synthesis.

A molecular model for DCBR recombination of λ proposes that terminase makes a DCB at *cos*. The duplex end is digested on one chain to give a single-stranded overhang which invades an uncut homolog (Figure 1). Appropriate nicks and ligations lead to the formation of a recombinant chromosome with hybrid DNA at the splice junction. The above model was proposed for Red recombination in *recA* cells (STAHL, KOBAYASHI and STAHL 1985; THALER, STAHL and STAHL 1987a). It takes into account the observed chemical polarity of the overlap (WHITE and FOX 1974; STAHL and STAHL 1974) and assigns roles to the Redα and Redβ proteins based on their known biochemical properties (LITTLE 1967; CARTER and RADDING 1971; RADDING *et al.* 1971; SRIPRAKASH *et al.* 1975; KMIEC and HOLLOMAN 1981; MUNIYAPPA and RADDING 1986).

That RecE and RecF are similar to Red is suggested by the biochemical activities of enzymes involved in the two pathways. The RecE protein is a 5′ → 3′ exonuclease, like λ's exonuclease (KUSHNER, NAGAISHI and CLARK 1974; GILLEN *et al.* 1977; JOSEPH and KOLODNER, 1983a,b). The RecF pathway is fully active in a *recBC sbcB sbcC* background (KUSHNER, NAGAISHI and CLARK 1972; LLOYD and BUCKMAN 1985; LUISI-DELUCA, LOVETT and KOLODNER 1989). The *sbcB* mutation inactivates exonuclease I (ExoI), a 3′ → 5′ exonuclease specific for 3′ overhangs, suggesting that a RecF pathway intermediate carries 3′ overhangs sensitive to ExoI (KUSHNER *et al.* 1971).

The RecBCD pathway of recombination operating in wild-type *E. coli* is, by definition, dependent on functional *recB*, *recC* and *recD* genes (reviewed in CLARK and LOW 1988). These are closely linked on the genetic map and encode the three subunits of the RecBCD enzyme [see TAYLOR (1988) for review]. In addition, recombination depends on the *recA* gene as well as on other genes associated with DNA metabolism (reviewed in SMITH 1987). The action of the RecBCD pathway on λ is seen when λ is mutant for the genes *red* and *gam*.

In the absence of a χ sequence, 5′GCTGGTGG (SMITH *et al.* 1981), exchanges are distributed uniformly along the length of the λ chromosome (STAHL *et al.* 1974). When χ is present in the proper orientation, exchanges show a maximum near χ and decrease in a gradient extending leftward (LAM *et al.* 1974; CHENG and SMITH 1989). These observations appear to reflect the following series of events (KOBAYASHI, STAHL and STAHL 1984): (i) terminase, the packaging enzyme, binds to and linearizes the λ chromosome at *cos*, generating cohesive ends; (ii) terminase remains bound at λ's left end and initiates packaging, which occurs in a polar fashion from left to right (as in Figure 1) (reviewed in FEISS and BECKER 1983); (iii) *cos*-cutting by terminase provides an asymmetric entry site for the RecBCD enzyme, which enters the λ chromosome at the right end and travels leftward along the chromosome; (iv) when RecBCD recognizes χ it carries out recombination with high probability near χ. The probability of exchange falls off with increasing distance leftward from χ. The mechanism by which χ and RecBCD stimulate recombination remains under active investigation (SMITH 1987; THALER and STAHL 1988; STAHL *et al.* 1990b).

A mutation in the *recD* gene results in a RecBC(D^-) enzyme which has lost exonuclease V (ExoV) activity but not recombination proficiency (AMUNDSEN *et al.* 1986; BIEK and COHEN 1986; CHAUDHURY and SMITH 1984; THALER *et al.* 1989). λ recombination in a *recD* mutant strain [RecBC(D^-) recombination] differs strikingly in several phenomenological aspects from recombination by the wild-type RecBCD pathway. Unlike the RecBCD pathway, RecBC(D^-) recombination occurs near the site of a DCB whether it be made by terminase at *cos*, or by a type II restriction enzyme at its recognition site. Among phage with unreplicated chromosomes, RecBC(D^-) recombination occurs preferentially near *cos*. Phage with replicated chromosomes show a more even distribution of exchanges. RecBC(D^-) recombination is also insensitive to the presence of χ. With respect to increased recombination at a DCB, a replication dependent

distribution of exchanges, and blindness to χ, RecBC(D^-) recombination is unlike the RecBCD pathway and bears close resemblence to the DCB pathways Red, RecF and RecE (THALER *et al.* 1989). RecBC(D^-) cells are hyper-rec (under χ^0 conditions) compared to wild-type (CHAUDHURY and SMITH 1984; THALER *et al.* 1989); recombination still requires the *recC* function (LOVETT, LUISI-Deluca and KOLODNER 1988; THALER *et al.* 1989) and so presumably is dependent on a RecBC(D^-) enzyme. The Rec^- phenotype of *recB* mutants that are polar on *recD* supports that view (AMUNDSEN *et al.* 1986).

In λ crosses blocked for DNA replication, the distribution of exchanges in *recD* cells is similar to that for rec^+ cells when λ carries χ^+D. χ^+D is located near the right end of λ and is recognized by the wild type enzyme soon after its entry into the λ chromosome. This observation along with the fact that *recD* mutants elevate recombination of χ^0 phage to the level experienced by χ^+ phage in a rec^+ host suggests one view of RecBC(D^-) and RecBCD mediated recombination: that the RecBC(D^-) enzyme is equivalent to a wild-type RecBCD enzyme that has seen χ and become activated for recombination (THALER *et al.* 1988, 1989). According to this picture, the function of χ is to inhibit the action of the D subunit responsible for ExoV activity by dissociating the D subunit from the BC part of the enzyme. The D-minus enzyme is activated for recombination, and continues to travel along the DNA, carrying out recombination with high probability (STAHL *et al.* 1990b).

A different view of RecBCD mediated recombination is based on the observation that RecBCD enzyme is observed to nick DNA at χ *in vitro* (PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985). Nicking occurs on the DNA chain carrying 5′GCTGGTGG four to six nucleotides 3′ of χ. Features of the cutting correlate well with χ action *in vivo*. Cutting is orientation dependent–in order for RecBCD to make the nick, it has to approach χ from 3′ → 5′ on the chain that is nicked. This is the same orientation of χ (defined with respect to *cos*) that is active *in vivo* (KOBAYASHI *et al.* 1982; STAHL *et al.* 1986). Additional correlations between χ activity *in vivo* and the nick observed *in vitro* have been made. The ability of a mutant or homologous RecBCD enzyme to respond to χ *in vivo* agrees with its ability to nick at χ *in vitro* (SMITH 1987). Similarly, the hot-spot activity of χ-like sequences *in vivo* is congruent with the relative amount of nicking they undergo *in vitro* (CHENG and SMITH 1984, 1987). These observations and properties of the RecBCD enzyme form the basis of a model (Figure 2) proposed in SMITH *et al.* (1984) in which RecBCD enters the DNA at *cos* and tracks from right to left, unwinding and rewinding the DNA. On seeing χ, the enzyme makes a nick on the *l* chain a few nucleotides 3′ to the χ sequence. The enzyme continues to unwind the DNA spinning out a 3′ ended single chain which invades a homologous duplex. The invasion leads to formation of a D-loop. The displaced chain is nicked, releasing a 3′ end which anneals to its complement on the aggressor molecule forming a Holliday junction intermediate carrying symmetric heteroduplex DNA. Resolution of the junction in either of two senses yields, respectively, a noncrossover (patch) product which carries a single-chain insertion heteroduplex on the *l* chain, or a crossover (splice) product with heteroduplex DNA at the splice junction, the overlap being that formed by 3′ overhangs.

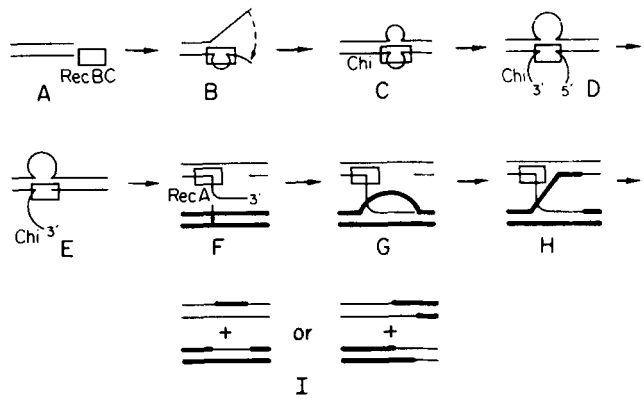


FIGURE 2.—Model for RecBCD-mediated recombination in which recombination is initiated by a nick at χ (from SMITH *et al.* 1984). RecBCD (called RecBC in the figure) enters the DNA at the right end and unwinds it (A–C). Nicking at χ (D) is followed by further unwinding (E) to release a 3′ ending single chain which invades (vertical arrow) a homologous molecule (F). The resulting D-loop is nicked at the right end (G) leading to the formation of a Holliday junction (H). Resolution of the junction in either of two senses gives a noncrossover (patch) product or a crossover (splice) product (I). Patches arise as single-strand insertions on the *l* chain. Splices have 3′ overlaps.

This work examines the chain polarity of recombinant heteroduplexes that have crossed over for flanking markers (splices) in the RecBCD, RecBC(D^-), RecF, and Red pathways. Chain polarity of splices was studied for two orientations of *cos*. We find that for all of the pathways a majority of the heteroduplexes have a structure consistent with an overlap of complementary 3′ ends to make a splice.

MATERIALS AND METHODS

Bacterial strains and phage mutations are listed in Tables 1 and 2, respectively. Approximate map locations of phage mutations are shown in Figure 3. Standard methods were used in the construction of phage strains (ARBER *et al.* 1983).

Phage crosses: Replication blocked crosses were conducted as described previously (STAHL *et al.* 1972b). Cells (5–10 ml) were grown at 26° in λ Tryptone (Difco) broth supplemented with yeast extract (0.01%) and vitamin B1 (20 μg/ml), to a density of 2×10^8 cells/ml. A phage mix containing each parent at $1–2 \times 10^9$/ml was made in λ Tryptone (Difco) broth. 1–2 ml of preheated phage mix was added to an equal volume of culture at 41.5° to give a

TABLE 1

***E. coli* strains and plasmids**

Designation	Description	Source/Reference
C600	SuII$^+$ *rec*$^+$	APPELYARD (1954)
594	Su$^-$	WEIGLE (1966)
QR48	SuII$^+$ *recA*	SIGNER and WEIL (1968)
QD5003	SuIII$^+$	GOLDBERG and HOWE (1969)
JC9387	Su$^-$ *recB21 recC22 sbcB15 sbcC201*	LLOYD and BUCKMAN (1985)
JC9937	Su$^-$ *rec*$^+$	A. J. CLARK
JM1	SuIII$^+$ *recB21 sbcA20*	STAHL *et al.* (1980)
JMB9	SuII$^+$ *recA56*	WOLD *et al.* (1982)
V227	SuII$^+$ *recC1001* (P2)	CHENG and SMITH (1989)
SMR34	*mutL216*::Tn*10* (λ *cos2*)	ROSENBERG (1987)
SMR88	SuII$^+$ *recD1009* Δ*hsdSM*$_k$ ($r_k^-m_k^-$)	ROSENBERG (1988)
M6001B	SuI$^+$	Laboratory collection
CES202	JC9387 thr$^+$ leu$^+$ *hsdR* ($r_k^-m_k^+$)	Laboratory collection
FS1461	C600 [pMB4]	Laboratory collection
FS1585	SuII$^+$ SuIII$^+$ *recD1009*	Laboratory collection
FS1585	SuII$^+$ SuIII$^+$ *recD1009*	STAHL *et al.* (1986)
FS1641	Su$^-$ *recD1009*	THALER *et al.* (1989)
FS2214	Su$^-$ *dnaBts22 malB*::Tn*9 mutL211*::Tn*5*	Laboratory collection
FS2268	JC9387 *dnaBts22 malB*::Tn*9* Δ*hsdSM*$_k$ ($r_k^-m_k^-$)	This work
FS2372	Su$^-$ *recD1014 hsdR* ($r_k^-m_k^+$)	This work
FS2519	CES202 *dnaBts22 malB*::Tn*9 mutL211*::Tn*5 hsdR* ($r_k^-m_k^+$)	This work
FS2565	Su$^-$ *recD1013 dnaBts22 malB*::Tn*9*	THALER *et al.* (1989)
FS2628	Su$^-$ *recD1009 mutL211*::Tn*5* [pJC1]	This work
FS2629	SuII$^+$ *recD1009 mutH471*::Tn*5*	This work
FS2924	FS2565 *mutL211*::Tn*5*	This work
SI121	CES202 *dnaBts22 malB*::Tn*9 mutH471*::Tn*5 hsdR* ($r_k^-m_k^+$)	This work
pMB4	Plasmid carrying the *Eco*RI restriction modification system and ampicillin resistance	BETLACH *et al.* (1976)
pJC1	*Eco*RI modifying plasmid with chloramphenicol resistance	NEWMAN *et al.* (1981)

FS2519, SI121 and FS2268 were derived from JC9387 by P1 transduction; FS2214 was derived from JC9937 by P1 transduction of *dnaBts22 malB*::Tn*9* and *mutL*::Tn*5*. All cross hosts are in an AB1157 background.

TABLE 2

λ Genetic elements

Name	Description	Source/Reference
Oam29	Amber in gene *O*: GC → TA transversion	RADICELLA, CLARK and FOX (1988)
Pam80	Amber in gene *P*: CG → TA transition	REISER (1983)
Pam3	Amber in gene *P*: CG → TA transition	REISER (1983)
cI26	Unconditional mutation in gene *cI*	MESELSON (1964)
cI857	Temperature sensitive mutation in gene *cI*	SUSSMAN and JACOB (1962)
Rts129	Temperature sensitive mutation in gene *R*	CAMPBELL and DEL CAMPILLO-CAMPBELL (1963)
Jts15	Temperature sensitive mutation in gene *J*	BROWN and ARBER (1964)
cos2	22-bp deletion of *cos* removing nicking site	KOBAYASHI *et al.* (1982)
cosML	φ80-λ hybrid *cos* inserted in leftward orientation at 47% from λ's left end	KOBAYSAHI *et al.* (1982)
bl453	*red*$^-$*gam*$^-$ deletion from *att* rightward	HENDERSON and WEIL (1975)
A^{21} (*hy33*)	21-λ hybrid allele of terminase carrying left end from phage 21; packaging specificity is for cos^{21}	FRACKMAN, SIEGELE and FEISS (1984)
Aam11	Amber mutation in gene *A*	CAMPBELL (1961)
bio1	Substitution right of *att*	HRADECNA and SZYBALSKI (1969)
int4	Unconditional *int* defective	GINGERY and ECHOLS (1967)
b2	6-kb deletion leftward from *att*	SZYBALSKI and SZYBALSKI (1979)
gam210	Amber mutation in *gam*	ZISSLER, SIGNER and SCHAEFFER (1971)
red3	Unconditional mutation in *redβ*; polar on *redα*	SIGNER and WEIL (1968)
nin5	5% deletion in right arm	FIANDT *et al.* (1971)

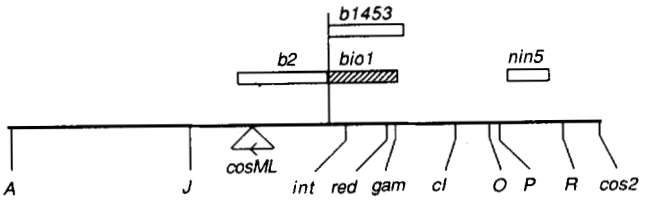


FIGURE 3.—Map of λ showing approximate location of genetic elements used in this study.

multiplicity of infection of 5–10 of each parent. The mixture was aerated by bubbling for 65 min. At the end of the cross, unadsorbed phage was removed by centrifugation. The pellet was resuspended in 2 ml broth. Cells were lysed with chloroform and lysozyme and the debris was spun down. Cross lysates were centrifuged in cesium formate equilibrium density gradients (refractive index adjusted to 1.3753–1.3775) in a SW50.1 rotor (Beckman) at 30,000 rpm for 18–42 hr. Fractions of 2 or 3 drops each were collected into 1 ml Tryptone broth. Fractions were surveyed by spotting on indicator plates by means of a glass capillary tube. They were then titered by plaque assay.

Heteroduplex analysis: *MutY selection scheme:* Phage from the respective fractions were adsorbed onto FS2628 (Su⁻ *recD mutL* [pJC1]) at 34° for 20 min, and the infected cells were plated onto a lawn of FS1461 (SuII⁺[pMB4]) cells at 37°. Well separated plaques were picked after an incubation of 8–12 hr. The plaques tended to be small and heterogenous in size, hence the long incubation (see below). The plaques were picked into 1 ml TM buffer. Single isolates were spotted by means of a glass capillary onto (i) FS2372 (Su⁻), (ii) JMB9 (SuII⁺ *recA*), and (iii) FS1585 (SuII⁺ SuIII⁺ *recD*), in that order. In the $A^{21}(hy33)$ crosses, JMB9 was omitted. Isolates spotting positive on FS2372 and on JMB9 (splices) were plated on FS1585, and single plaques (around 15–30 from each isolate) were stabbed with toothpicks onto Su⁻, SuI⁺, SuIII⁺ and SuII⁺ (or SuII⁺ SuIII⁺) strains, the last at 37° (for $A^{21}(hy33)$ crosses) or 41° (for *Jts* and *Rts* crosses). *Pam3* and *Pam80* were distinguished as follows: *Pam3* phage grew on SuI⁺ and SuII⁺ but not on SuIII⁺; *Pam80* phage grew on SuII⁺ and SuIII⁺ but not on SuI⁺. Isolates that contained at least 30% P^+ phage and either *Pam3* or *Pam80*, but not both, were taken to have arisen from mismatch correction of a *P3/P80* heteroduplex to give P^+ information on the transcribed *r* DNA chain. Plaques containing less than 30% P^+ phage were classified as homozygous mutant and were considered to have leaked through the selection. Typically such plaques contained no P^+ phage. P^+ phage when present in a plaque were generally in a majority so that the 30% value, chosen somewhat arbitrarily early in the work and maintained throughout, was not a critical factor in the analysis. Plaques containing both *Pam3* and *Pam80* were not observed.

Very short patch (VSP) analysis scheme: The cross host was SI121 (Su⁻ *recBC sbcB sbcC dnaBts mutH hsdR*). The input phage were unmodified for EcoK. The progenies from the crosses were plated on FS2629 (SuII⁺ *recD mutH*) at 37°. Only phage that had matured in the cross host and thereby become modified for EcoK were able to form a plaque. This reduced the plating of unabsorbed phage to an extent that greater than 90% of the plaques examined came from phage that had matured through the cross host. Young (5–7 hr), well separated plaques were picked into 1 ml TM buffer and spotted onto Su⁻ and SuII⁺ strains. Isolates that were positive on Su⁻ were plated out to give single plaques. Around 20–30 plaques (both *c* and c^+ where possible) from each isolate were stabbed onto lawns of Su⁻, SuI⁺, SuIII⁺ and SuII⁺, in that order. *Pam3* and *Pam80* were distinguished as above. Plaques containing P^+ and either *Pam3* or *Pam80* phage, but not both, were taken to have arisen by mismatch correction of a *P3/P80* heteroduplex. P^+ containing plaques that also contained both *Pam3* and *Pam80* phage were considered to have given P^+ phage by recombination following replication and were classified as *P3/P80*. Plaques containing less than 30% P^+ phage and only one mutant allele were classified as homozygous mutant just as in the MutY scheme; P^+ phage in these plaques could have arisen by recombination with another phage during growth of the plaque or could come from reversion during growth of the plaque on the *mutH* indicator. Typically such plaques contained less than 5% P^+ phage, whereas P^+ phage considered to arise by mismatch correction were generally in a majority.

Wild-type mismatch repair: Phage from the respective fractions were adsorbed onto a plating culture of strain FS2372 (Su⁻) for 20 min at 34° and plated onto a lawn of C600 (SuII⁺) at 37° to give around 20 plaques per plate. Young (5–7 hr), well separated plaques were picked into 1 ml TM buffer and spotted onto Su⁻ and SuII⁺ strains. Plaques that were positive on Su⁻ were plated on C600 to give single plaques. Around 30 plaques from each isolate were poked onto Su⁻ and Su⁺ to identify amber mutants. These were then tested for *Pam3*, *Pam80* and *Oam29* alleles by spotting an isolate onto lawns of (i) Su⁻; (ii) Su⁻ seeded with *Pam3*; (iii) Su⁻ seeded with *Pam80*; and (iv) Su⁻ seeded with *Oam29*. Separate capillaries were used for spotting onto *Pam3*, *Pam80* and *Oam29* plates. As in the above schemes, where the isolate contained both *c* and c^+ plaques, both were poked onto the indicator lawns. The principles for assigning heteroduplex genotypes were the same as in the above schemes.

Construction of artificial heteroduplexes: High titer phage preparations were made from plate stocks of $A^{21}(hy33)$ *bio1 cI26 Pam3* and $A^{21}(hy33)$ *bio1 Pam80*. Strand separation was according to the method of SZYBALSKI *et al.* (1971). Renaturation was according to the method of MESELSON and YUAN (1968): 10–20 μl of each preparation of separated chains (at a DNA concentration of approximately 50 μg/ml) were self-annealed or annealed with an equal volume of the complementary chain preparation and packaged *in vitro*. The packaged phage were then titered. Separated chains of 99% or greater purity as measured by this assay were used for making artificial heteroduplexes. *In vitro* packaging was as described in ROSENBERG (1987) using a *mutL* packaging extract. Artificial heteroduplex phage were analyzed by the MutY and VSP schemes just as were the crosses.

***Aam11* allelism tests:** Plaques to be tested were picked into 1 ml TM buffer and spotted onto (i) Su⁻, (ii) SuII⁺ and (iii) Su⁻ seeded with *Aam11* phage. Those spotting positive on (ii) and (iii) carried the $A^{21}(hy33)$ allele. Those spotting positive on (ii) and negative on (iii) carried the *Aam11* allele.

RESULTS

Experimental strategy and design: The experimental approaches we have used to study heteroduplex chain polarity are based on properties of the *O* and *P* genes. These genes specify functions essential for the replication of λ DNA and are transcribed from the *r* chain (reviewed in FRIEDMAN and GOTTESMAN 1983). The selection of wild-type information from the *r* chain was used by WHITE and FOX (1974) to study chain polarity in the Red pathway. In addition, the analysis relies on properties of particular alleles of the *P* gene and on the ability of mismatch repair

systems to rectify heteroduplex DNA. Some of these properties and principles have been used to study chain polarity in the Red (WHITE and FOX 1974; STAHL and STAHL 1974) and RecBCD (ROSENBERG 1987, 1988) pathways.

Genes that affect mismatch repair in *E. coli* have been identified (reviewed in RADMAN 1988). Combinations of alleles define several mismatch repair systems, some with overlapping genetic requirements. By a selective use of mutations, one or another mismatch repair system can be brought to act on a given set of mismatches, processing them in a manner characteristic of that system. We have used mutations in the *mutL* and *mutH* genes that lead to processing of the *P3*/+ and *P80*/+ mismatches by the MutY (NGHIEM *et al.* 1988; RADICELLA, CLARK and FOX 1988) and very short patch (VSP) (LIEB, ALLEN and READ 1986) systems, respectively. Both *Pam3* and *Pam80* are C·G to T·A transitions (REISER 1983) in a sequence context (5′CCAG → 5′CTAG) that allows correction by the VSP repair system. The MutY system corrects G·A and C·A mispairs to G·C and C·G, respectively (RADICELLA, CLARK and FOX 1988), and the VSP system corrects G·T to G·C (LIEB 1983; JONES, WAGNER and RADMAN 1987). Two heteroduplex analysis schemes based on these systems were employed and are discussed below.

When the cross host and Su⁻ indicator are *mutL*, mismatch correction by MutY is operative (RADICELLA, CLARK and FOX 1988), but the contributions from the long patch and very short patch repair systems are greatly reduced (RADMAN 1988). In the MutY scheme (Figure 4), the *P3/P80* phage enters an Su⁻ *mutL* cell and is unable to replicate unless it becomes P^+ by mismatch correction. When this happens so as to give P^+ information on the chain from which *P* is transcribed (the *r* chain), the phage can make P^+ protein and replicate. The P^+ allele is dominant so that the initial burst contains not only P^+ phage but also phage derived from the mutant allele that was present on the *l* chain of the heteroduplex. When plated on an Su⁺ lawn, the Su⁻ infective center will give a plaque that contains both types of phage. By inference the mutant allele represented in the plaque must have been present on the *l* chain of the heteroduplex. This information allows the assignment of a chemical polarity to the splice junction in the recombinant (WHITE and FOX 1974).

We noted that plating of *P3/P80* artificial heteroduplexes as well as phage from the crosses on the MutY scheme gave plaques that were small and heterogeneous in size. Plating of P^+ phage under the same conditions gave larger and more uniform plaques. Similar observations have been made by other investigators on the plating of artificial heteroduplexes of genes *O* and *P* on a Su⁻ *mutL* indicator

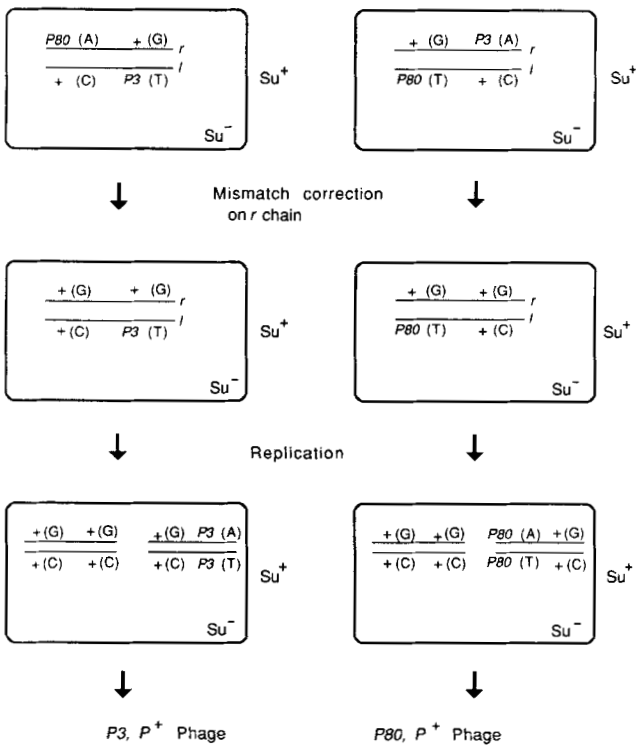


FIGURE 4.—The MutY scheme for analysis of heteroduplex chain polarity. Principles and details of the method are described in MATERIALS AND METHODS and in the *Experimental strategy and design* section of RESULTS. The phage infects an Su⁻ *mutL* cell which is plated on an Su⁺ lawn. The *Eco*RI restriction-modification system was used to ensure that phage first go through a cycle of growth on the indicator FS2628 (Su⁻ *mutL recD* [pJC1]) and thereby become modified for *Eco*RI before growth on the *Eco*RI restricting lawn of FS1461 (SuII⁺[pMB4])cells.

(M. S. FOX, personal communication). This suggests that the MutY system is slow acting and that the correction event that gives P^+ on the *r* chain often occurs late in the growth of the bacterial lawn.

Under Su⁻ *mutL* conditions, correction to P^+ on the *r* chain can occur by the action of the MutY system. Because the long patch and very short patch repair systems have been inactivated by *mutL*, the mutant *P* allele on the *l* chain frequently escapes correction, so that many of the *P*+ containing plaques also contain one of the mutant alleles (Table 3). Under *mutH* conditions, both VSP and MutY repair systems are present. However VSP acts more rapidly on the heteroduplex than does MutY. When the *mutH* indicator is Su⁺, there is no selection for the heteroduplex to be maintained (*i.e.*, remain unreplicated) until mismatch corrected to P^+ as in the MutY scheme, so that the MutY system seldom acts on the heteroduplex and one sees predominantly the action of VSP (RAPOSA and FOX 1987).

VSP repair operates differently from MutY. When the long patch mismatch repair system has been specifically inactivated, as in a *mutH* or *uvrD* strain, the action of VSP repair on *P3/P80* heteroduplexes is most apparent (RAPOSA and FOX 1987). RAPOSA and

TABLE 3

Correction of artificial *P3/P80* heteroduplex phage

Genotype plaque	Artificial heteroduplex genotype: Type I (+ *P80* + / *c* + *P3*) MutY	Type I VSP	Type II (*c* + *P3* / + *P80* +) MutY	Type II VSP
+ *P80* / *c* +	13	0	0	10
c P3 / + +	0	14	13	1
+ *P3* / + +	0	0	0	1
c + / + +	7	2	1	0
c P3 / + *P80*	0	23	0	69
+ *P3* / + *P80*	0	1	0	0
c P3 / *c P80*	0	2	0	0
c P80 / + *P80*	1[a]		0	0
c + / *c* +	1	2	0	2
+ + / + +	1	10	3	1
+ *P80* / + *P80*	3[a]	3	0	8

Artificial heteroduplex phage were made by annealing of complementary strands from A^{21} (*hy33*) *bio1 cI26 Pam3* and A^{21} (*hy33*) *bio1 Pam80* phage. Phage were plated through the MutY selection scheme, and individual plaques were picked and analyzed. A total of 26 plaques for type I and 17 for type II heteroduplexes were analyzed by the MutY scheme. For the VSP scheme, artificial heteroduplex phage were plated on FS2629 (SuII$^+$ *recD mutH*). Plaques were picked and analyzed as described in MATERIALS AND METHODS. One hundred and seventy-nine plaques were picked and tested for type I and 361 for type II heteroduplexes. Only those isolates spotting positive on an Su$^-$ host (58 for type I and 92 for type II) are included in the data.

[a] These genotypes were considered to have leaked through selection by the Su$^-$ indicator.

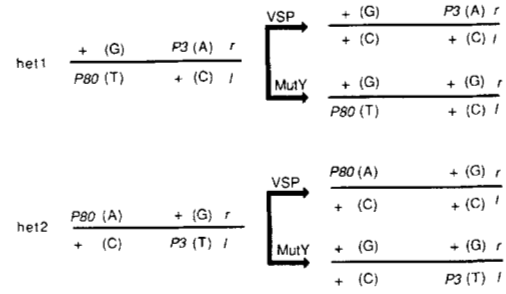


FIGURE 5.—Correction of heteroduplexes by the MutY and VSP repair scheme for analysis of chain polarity. See MATERIALS AND METHODS and *Experimental strategy and design* sections of RESULTS for details and explanation of the methodology. Correction to P^+ in the VSP scheme occurs on the *l* chain. In the MutY scheme, correction occurs on the *r* chain.

Fox observed that *P3(r)/P80(l)* is corrected to *P3/+*, while *P80(r)/P3(l)* is corrected to *P80/+*. Thus VSP repair corrects the mutant allele on the *l* chain to P^+ and leaves the mutant allele on the *r* chain unrepaired (Figure 5). This being the case, the analysis involves a screen and not a selection for correction, making it considerably less efficient.

Correction of artificial heteroduplexes: Artificial *cI26 Pam3*/*cI*$^+$Pam80 heteroduplex phage were made as described above and subjected to heteroduplex analysis by the MutY and VSP schemes. Heteroduplexes were A^{21} (*hy33*) *bio1* to correspond with the genotype analyzed in the experimental crosses described in the next five sections. The results are shown in Table 3. The *P3(r)/P80(l)* heteroduplexes were corrected only to *+/P80* by the MutY scheme. No correction to *P3/+* was found (13:0). For the *P80(r)/P3(l)* heteroduplexes, correction was to *+/P3* (13:0). Correction by VSP was on the other chain; thus *P3(r)/P80(l)* was corrected to *P3/+* (14:0), and *P80(r)/P3(l)* was corrected predominantly to *P80/+* (10:2). These results validate the methodology for heteroduplex analysis by the two schemes and are in agreement with the observations of RAPOSA and FOX (1987).

Design of crosses for analysis of RecF heteroduplex chain polarity: It was anticipated that splices formed via the RecF pathway would involve 3′ overhangs (see Introduction). To test this notion we conducted crosses of the following form:

Cross I		Cross II
A^{21}(*hy33*) *bio1 Pam80*		*Aam11 b2bio1 cI Pam3*
×	and	×
A^{21}(*hy33*) *bio1 cI Pam3*		*Aam11 b2bio1 Pam80*

The bio1 substitution eliminated λ recombination functions. The *Aam11* allele blocks *cos* cutting on the lower phage, while *cos* cutting can occur on the phage carrying the heterospecific *cos* and terminase substitution A^{21}(*hy33*) (HOHN 1975; FRACKMAN, SEIGLE and FEISS 1984). Hence, the recombination-stimulating double-strand break should occur only on the A^{21}(*hy33*) parent in each cross. (Confirmation that the *Aam11*/A^{21}(*hy33*) allele pair works as advertised is presented in the next section.) According to the model (Figure 1), exonuclease digestion in a 5′ → 3′ direction and subsequent steps should lead to the formation of a recombinant with one type of heteroduplex overlap, that in which the uncut *Aam11* parent contributes on the *r* chain, which ends 5′ at λ's right end.

In the MutY and VSP heteroduplex analyses, unadsorbed phage were eliminated by crossing unmodified parent phage in r_k^- m_k^+ hosts and plating the yields on r_k^+ m_k^+ strains.

Evidence that use of the allele pair A^{21}(*hy33*) and *Aam11* defines the phage that initiates recombina-

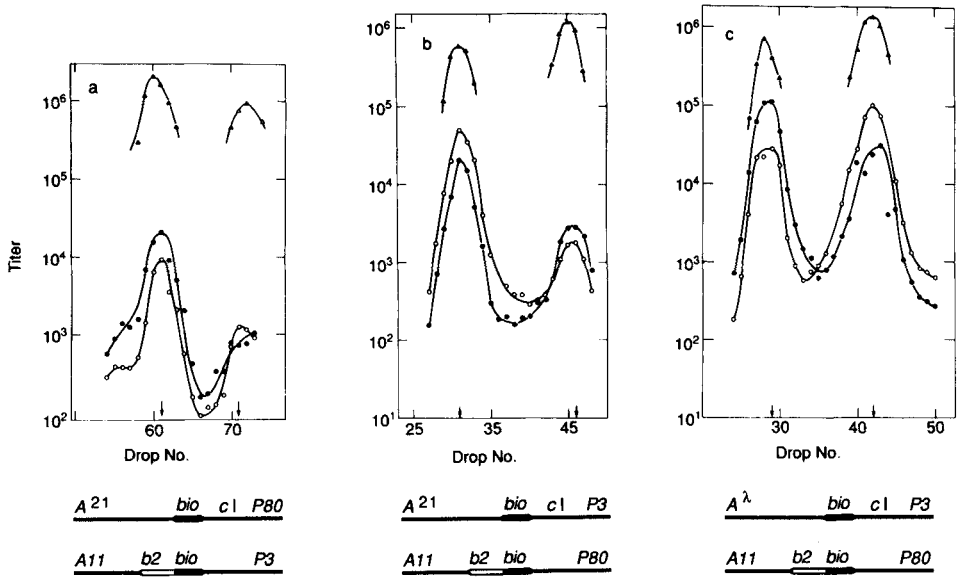


FIGURE 6.—Density gradients of crosses conducted under replication blocked conditions in the host FS2519 [Su⁻ *recBC sbcB sbcC dnaB mutL hsdR* ($r_k^-m_k^+$)], which is nonrestricting but modifying ($r_k^-m_k^+$). Input phage were unmodified. The heavy and light peaks correspond to the *bio1* and *b2bio1* phage, respectively. Phage from these regions were tested for their *A* allele, as described in MATERIALS AND METHODS. Arrows indicate fractions from which phage were plated for heteroduplex analysis by the MutY protocol and for *Aam11* allelism tests. Similar crosses were carried out in SI121 (Su⁻ *recBC sbcB sbcC mutH hsdR* ($r_k^-m_k^+$) for heteroduplex analysis by the VSP shceme (not shown). (○) c^+ phage on FS1585 (SuII⁺ SuIII⁺ *recD*); (●) *c* phage on FS1585; (△) c^+ phage on FS2501; (▲) *c* phage on FS2501 (SuII⁺ *recD hsdSM*$_k$).

tion: To assure ourselves that λ A^{21}(*hy33*) contributes poorly to the packaging of λ cos^{λ} in these experiments, we compared the packaging of *Aam11* chromosomes by A^{21}(*hy33*) with packaging by A^+. The two parents in each cross (Figure 6) differ in density by virtue of the *b2* deletion. The lysates from the crosses were fractionated by equilibrium density centrifugation, and the fractions were titered (Figure 6). The input phages in the crosses were unmodified for K12. The cross host was nonrestricting but modifying for K12. The cross lysate therefore contained phage that had gone through the cross host and became modified for K12 as well as unadsorbed phage that were unmodified. In the crosses of Figure 6, aand b, the number of modified phage in the heavy peak as titered on a Su⁺ K12 restricting indicator (FS1585) is approximately 20 times greater than the number of modified phage in the light peak. The heavy peak corresponds to the A^{21}(*hy33*) parent and the light peak to the *Aam11* parent. In the cross shown in Figure 6c, where the heavy phage carried the A^+ allele of terminase, the numbers of modified phages in the two peaks are equal. These observations are consistent with the previous finding (FRACKMAN, SIEGELE and FEISS 1984) that A^{21}(*hy33*) compared to A^+ complements *Aam11* poorly. Allelism tests for *Aam11* (Table 4) showed that in the cross of Figure 6c a greater proportion of modified phage in both the heavy and light peaks carried *Aam11* as compared to crosses in (a) and (b), further supporting this conclusion.

MutY heteroduplex analysis: Replication blocked crosses were conducted in strain FS2519 [Su⁻ *recBC sbcB sbcC dnaBts mutL hsdR* (r_k^- m_k^+)] cells expressing the RecF pathway and carrying *mutL*. Phage from the heavy peaks of Figures 6a and 6b were analyzed as

TABLE 4

***Aam11* allelism tests of cross progeny**

Plaque genotype	Cross I		Cross II		Cross III	
	Heavy peak	Light peak	Heavy peak	Light peak	Heavy peak	Light peak
A21 c	21	29	79	20		
A^{21} +	54	4	20	25		
A^{21} *c*/+	3	2	1	0		
Aam11 c	0	41	0	19	5	15
Aam11 +	0	24	0	34	0	26
Aam11 c/+	0	0	0	2	0	1
A^+ *c*					30	2
A^+ +					14	6
A^+ *c*/+					1	0
Total *Aam11*	0	65	0	55	5	42
Total tested	78	100	100	100	50	50

Crosses were I: A^{21} (*hy33*) *bio1 Pam80* by *Aam11 b2bio1 cI26 Pam3*; II: A^{21} (*hy33*) *bio1 cI26 Pam3* by *Aam11 b2bio1 Pam80*; III: A^+ *bio1 cI26 Pam3* by *Aam11 b2bio1 Pam80*. The cross host was FS2519 (Su⁻ *recBC sbcB sbcC dnaBts mutL*). Phage were unmodified for K12. Phage from the respective peaks (Figure 6) were plated on FS1585 (SuII⁺ SuIII⁺ *recD*). Plaques were picked and tested for their *A* allele. Plating efficiencies of unmodified *Aam11 b2bio1 Pam80* and *Aam11 b2bio1 cI26 Pam3* phage on FS1585 were 1.0×10^{-3} and 4.6×10^{-4}, respectively, relative to SMR88 (SuII⁺ *recD hsdSM*$_k$ ($r_k^-m_k^-$)).

described above, and the results are shown in Table 5. In cross I, 18 *P80*/+ and 4 *P3*/+ heteroduplexes were found. When *Pam3* and *Pam80* markers were reversed (cross II), 12 *P3*/+ and 1 *P80*/+ heteroduplexes were found. In each case, the majority of heteroduplexes had a contribution from the uncut *Aam11* parent on the *r* chain, as expected (Figure 7a).

VSP heteroduplex analysis: A set of crosses identical to the one above was carried out in a RecF pathway host [SI121) that was *mutH* (Su⁻ *recBC sbcB sbcC dnaBts mutH hsdR* (r_k^- m_k^+)], and phage from the

TABLE 5

Allele analysis of plaques from A^{21} (*hy33*) by *Aam11* RecF pathway crosses

Plaque genotype	MutY		VSP	
	Cross I[a]	Cross II	Cross I[b]	Cross II
$\frac{c\ P3}{+\ +}$	4	6	11	3
$\frac{c\ P3}{c\ +}$	0	6	2	1
$\frac{+\ P3}{+\ +}$	0	0	8	0
$\frac{+\ P80}{c\ +}$	5	1	2	6
$\frac{+\ P80}{+\ +}$	12	0	2	1
$\frac{c\ P80}{c\ +}$	1	0	1	3
$\frac{c\ P3}{+\ P80}$	0	0	3	3
$\frac{c\ P3}{c\ P80}$	0	0	2	3
$\frac{+\ P3}{+\ P80}$	0	0	1	0
$\frac{c\ +}{+\ +}$	5	1	1	2
$\frac{c\ +}{c\ +}$	3	1	3	9
$\frac{+\ +}{+\ +}$	8	0	4	5
$\frac{+\ P80}{+\ P80}$	2	0	1	0
$\frac{+\ P3}{+\ P3}$	0	11	0	2

Phage from the heavy peak of crosses shown in Figure 6 were analyzed by the MutY and VSP schemes. Most of the plaque genotypes could be explained as arising from correction of heteroduplexes derived from overlap of parental DNA chains. Of these, in the MutY scheme, 18 were corrected to *P80*/+ and 4 to *P3*/+ in cross I; in cross II, 12 were corrected to *P3*/+ and 1 to *P80*/+. In the VSP scheme, 21 were corrected to *P3*/+ and 5 to *P80*/+ in cross I; in cross II, 10 were corrected to *P80*/+ and 4 to *P3*/+. Homoduplex *P3* and *P80* phage observed in the MutY scheme were considered to have escaped selection. In the MutY scheme, 42 plaques from cross I and 26 from cross II were analyzed. Approximately 500 plaques were picked and tested in cross I and 450 in cross II in the VSP scheme; only those isolates that spotted positive on Su^- were analyzed further.

[a] One $\frac{c\ P80}{+\ +}$ plaque and one $\frac{+\ P3}{c\ +}$ plaque were also found.

[b] Three plaques had the genotype $\frac{+\ P3}{c\ P80}$. These genotypes cannot be explained as arising from correction of a heteroduplex derived from overlap of parental DNA chains and are considered to come from multiple splice events.

heavy peak were analyzed by the VSP scheme. The results are shown in Table 5. In cross I, 21 *P3*/+ and 5 *P80*/+ heteroduplexes were found. In cross II the numbers were 10 *P80*/+ and 4 *P3*/+. In each case, the uncut *Aam11* parent again contributes mostly to the *r* chain of the recombinant.

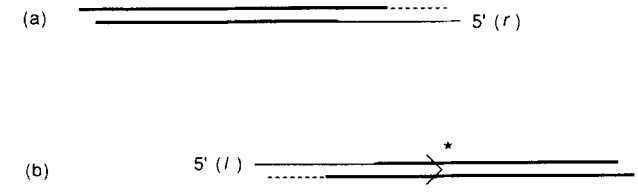


FIGURE 7.—Structures of splices according to the Red model for *cos*-stimulated exchange (Figure 1) in the case of (a) standard *cos*; (b) central leftward *cos*. Phage chromosomes are drawn as they would appear in the phage particle. The thin line indicates minority contribution from the parent that does not undergo the recombinogenic double-chain break. Recombinant phage (a) arises by cutting of standard *cos*, which is oriented rightward. Terminase remains bound at the left end of the linearized phage, preventing recombination there. The recombinant that arises at the right end (as in Figure 1) is packaged into a prohead that has bound to the terminase molecule responsible for cutting the *cos* of the heavy-lined phage. This same terminase molecule makes the cut at the right end that completes the packaging of the recombinant. Recombinant phage (b) arises as does phage (a) except that standard *cos* is inactivated (*) and events are initiated and completed at a *cos* site that is cloned in reverse orientation in the middle of the standard λ map.

TABLE 6

Allele analysis of plaques from A^{21} (*hy33*) by *Aam11* crosses plated on the Mut$^+$ selection scheme

Plaque genotype	Cross I	Cross II
$\frac{c\ P3}{+\ +}$	0	5
$\frac{c\ P3}{c\ +}$	1	11
$\frac{+\ P3}{+\ +}$	0	1
$\frac{+\ 029}{c\ +}$	10	7
$\frac{+\ 029}{+\ +}$	4	2
$\frac{c\ 029}{c\ +}$	1	3
$\frac{c\ +}{+\ +}$	5	9
$c\ +$	62	93
$+\ +$	16	5

Crosses were I: A^{21} (*hy33*) *bio1 Oam29 Pam80* by *Aam11 b2bio1 cI26 Pam3*; II: A^{21} (*hy33*) *bio1 cI26 Pam3* by *Aam11 b2bio1 Oam29 Pam80*. Lysates were centrifuged as in Figure 6. Phage from the heavy peak were analyzed by the MutY selection scheme.

Heteroduplex analysis with wild-type mismatch repair: Replication blocked crosses (Table 6) were conducted in FS2268 [Su^- *recBC sbcB sbcC dnaBts* $\delta hsdSM_k$ (r_k^- m_k^-)], a RecF pathway host wild type for mismatch repair genes. The lysate was centrifuged in

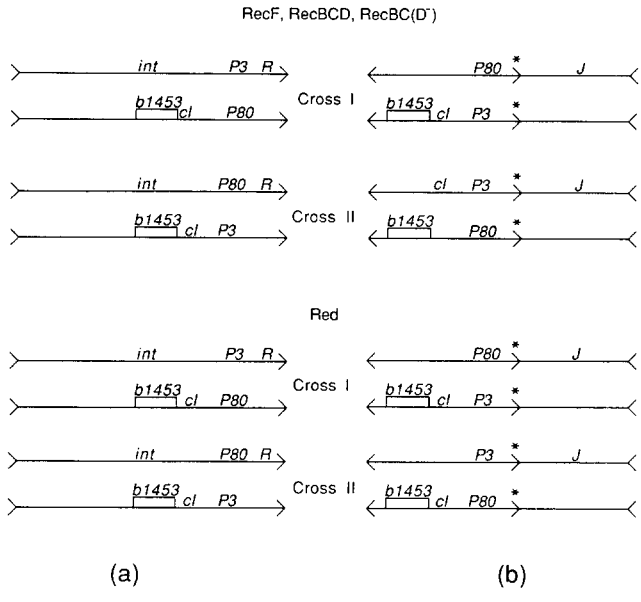


FIGURE 8.—Crosses to examine chain polarity of splices in the Red, RecF, RecBCD and RecBC(D⁻) pathways for (a) standard *cos*, and (b) *cos ML*. In (a) the flanking markers were *Rts129* and *b1453*. Splices were identified as R^+ phage that did not inherit the deletion. Flanking markers in (b) were *Jts15* and *b1453*. Splices were those that inherited J^+ from the heavy parent extending leftward from *cos*ML and had crossed over before *b1453* to lose the deletion. For the RecF, RecBCD and RecBC(D⁻) crosses, the partner to the *b1453* phage carried *red3 gam210*. All phage carried the *nin5* deletion. (>*) *cos2*; (<) *cos*ML.

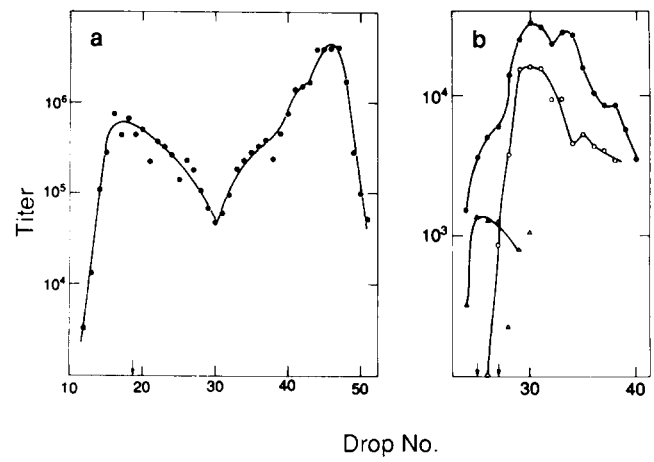


FIGURE 9.—Density gradients of crosses in Figure 8, a and b. Crosses were conducted under conditions restrictive for DNA synthesis in a *dnaBts* host at 41.5°. Shown are crosses to look at the RecF pathway done in strain FS2519: (a) cross I; (b) cross II. Similar graphs were obtained for crosses to look at the Red, RecBCD and RecBC(D⁻) pathways (data not shown). (a) P^+ splices are those that do not inherit *b1453* and are R^+; *b1453*$^+$ phage were identified as being able to grow on a *recA* indicator; the *R* allele was determined by replating the contents of the plaque and poking single plaques on FS1585 at 41°. (b) J^+ *b1453*$^+$ splices band at a position 3–6 fractions heavier than the density labeled parent. Phage from this region were plated for heteroduplex analysis by the MutY protocol. Plaques were picked and analysed, checking the state of the *J* allele at 41° and of *b1453* on a *recA* indicator. Heteroduplex platings were at 37°. Titers are (●) on FS1585 (SuII$^+$SuIII$^+$*recD*) at 32°; (○) on V227 [SuII$^+$ *recC1001* (P2)] at 37°; (△) on JMB9 (SuII$^+$ *recA*) at 32°.

a cesium formate gradient, and the heavy peak was analyzed as described above. The markers used for heteroduplex analysis are the same as those used in previous studies on the Red pathway by WHITE and FOX (1974) and by STAHL and STAHL (1974). The results shown in Table 8 lead to conclusions similar to those in the above analyses. In cross I, 15 *O29/+* and 1 *P3/+* heteroduplexes were found; in cross II, 17 *P3/+* and 12 *O29/+* heteroduplexes were observed. The lack of *P80/+* heteroduplexes and disparity in the bias between the two crosses are rationalized in the DISCUSSION.

Heteroduplex analysis of splices for Red, RecBCD, RecBC(D⁻) and RecF: Replication is blocked in the above experiments. Under these conditions dimerization is required for packaging of the phage chromosome (STAHL *et al.* 1972a). Thus, it is likely that most of the recombinants analyzed represent splices. However, some heteroduplexes could be noncrossover products (patches) in which maturation occurred by virtue of a second, independent recombination event leading to a splice. To reduce the contribution form such recombinants and to compare the Red, RecBCD RecBC(D⁻), and RecF pathways we carried out crosses diagrammed in Figure 8a. The *Rts* parent is density labelled with ^{13}C and ^{15}N isotopes; the other parent carries the *b1453* deletion. Splices are identified as R^+ recombinants that do not carry the deletion. It was brought to our attention during the course of this work that mutants of *recF* and *recO* are complemented by the *nin* region of λ (JAMES SAWITZKE, personal communication). To ensure that we are studying only *E. coli*'s RecF pathway, we incorporated the *nin5* deletion in both phage in these and subsequent experiments. The cross lysates were centrifuged and the fractions titered (Figure 9a). Splices are found in the parental heavy phage peak. Phage from the heavy peak were plated for analysis by the MutY protocol, and the results are shown in Table 7. In cross I for the Red pathway we saw 7 *P3/+* and 2 *P80/+* heteroduplexes; in cross II we saw 6 *P80/+* and 1 *P3/+*. For the RecF pathway, the results are similar: 11 *P3/+* and 0 *P80/+* in cross I; 5 *P80/+* and 2 *P3/+* in cross II. For the RecBCD pathway we observed 5 *P3/+* and 2 *P80/+* heteroduplexes in cross I; in cross II we found 8 *P80/+* and 0 *P3/+* heteroduplexes. For RecBC(D⁻), in cross I we found 5 *P3/+* and 0 *P80/+* heteroduplexes and in cross II we saw 8 *P80/+* and 3 *P3/+* heteroduplexes. The minority material contribution from the parent that, according to the model for Red, does not undergo the recombinogenic DCB tends to be on the 5′ ending *r* chain. For RecBCD this is also the polarity predicted by the model proposed in SMITH *et al.* (1984) and shown in Figure 2.

Role of *cos* in the formation of heteroduplex

TABLE 7

Splice polarity by MutY scheme for *cos*$^+$ *nin5* crosses for red, recF, recBCD and recBC(D$^-$)

Plaque genotype	Red cross no. I	Red cross no. II	RecF cross no. I	RecF cross no. II	RecBCD cross no. I	RecBCD cross no. II	RecBC(D$^-$) cross no. I	RecBC(D$^-$) cross no. II
+ *P3* / *c* +	7	0	8	1[a]	3	0	3	1[a]
c *P3* / + +	0	1	0	1	0	0	0	2
+ *P3* / + +	0	0	3	1	2	0	2	0
c *P3* / *c* +	0	0	0	0	0	0	0	1
c *P80* / + +	2	0	0	0	2	0	0	0
+ *P80* / *c* +	0	5	0	5	0	6	0	4
+ *P80* / + +	0	1	0	0	0	2	0	3
c *P80* / *c* +	0	0	0	0	0	0	0	1
c + / + +	11	10	3	4	0	2	0	2
+ + / + +	11	4	2	6	11	2	5	3
c + / *c* +	15	1	8	2	10	7	4	2

Crosses are diagrammed in Figure 8a. Lysates were centrifuged and phage from the heavy peak as in Figure 9 were plated for heteroduplex analysis by the MutY scheme. Single plaques were picked into buffer and their genotypes were determined as described in MATERIALS AND METHODS. Only P^+ containing phage that had recombined for flanking markers, *i.e.*, *b1453*$^+$ R^+ were analyzed.

[a] This genotype was considered to represent a multiple splice event (see Table 5 legend).

splices: All the above experiments have used phage with *cos* in its normal position. According to the model for Red (Figure 1), if *cos* were inverted and relocated to the left of *P*, 5′ → 3′ exonuclease digestion into *P* would occur on the *l* chain, so that the minority material contribution would now be found on the *l* chain (Figure 7). We tested this prediction for Red and RecF by means of crosses shown in Figure 8b. The *b1453* phage was density labelled, and the other phage carried *Jts*. Splices were located among total phage as a shoulder, or, in some crosses, as a distinct peak on the heavy side of the heavy parental phage peak. This corresponds to phage that inherit most of their DNA from the heavy parent while inheriting the *red gam* region, which is deleted in the heavy phage, from the light parent. In Figure 9b, the position of the *J*$^+$ *b1453*$^+$ recombinant peak coincides with the shoulder. Phage from this region of the gradient were

TABLE 8

Splice polarity by MutY scheme for *cos2 cosML nin5* crosses

Plaque genotype	Red cross no. I	Red cross no. II	RecF cross no. I	RecF cross no. II	RecBCD cross no. I	RecBCD cross no. II	RecBC(D$^-$) cross no. I	RecBC(D$^-$) cross no. II
+ *P80* / *c* +	11	0	11	0	11	4	11	4
+ *P80* / + +	1	0	4	0	0	0	2	0
c *P80* / *c* +	0	0	1	0	0	0	0	0
c *P80* / + +	0	1	0	0	0	0	0	0
c *P3* / + +	0	0	2	10	1	17	0	7
+ *P3* / *c* +	0	12	0	0	0	0	0	0
+ *P3* / + +	0	4	0	0	0	0	1	0
c *P3* / *c* +	0	0	1	1	1	3	0	3
c + / + +	8	1	2	0	3	2	3	1
c + / *c* +	1	1	7	0	1	1	0	1
+ + / + +	3	0	1	2	1	5	3	4

Crosses are diagrammed in Figure 8b. *J*$^+$ *b1453*$^+$ phage from the heavy peak (Figure 9b) were analyzed by the MutY selection/ scheme.

plated out for heteroduplex analysis by the MutY protocol. The results are shown in Table 8. For Red, in cross I we found 12 *P80*/+ and 0 *P3*/+ heteroduplexes; in cross II there were 16 *P3*/+ and 1 *P80*/+. For RecF, we saw 16 *P80*/+ and 3 *P3*/+ heteroduplexes in cross I; in cross II we observed 11 *P3*/+ and 0 *P80*/+. The results for Red and RecF are similar– in each case, for both marker configurations, the minority material contribution is now found predominantly on the *l* chain.

For the RecBCD pathway, the model shown in Figure 2 predicts that inverting and relocating *cos* to the left of *P* will also give 3′ splices with minority material contribution on the *l* chain. For recombination in a *recD* strain, we have no prediction. We examined the effect of changing the location and orientation of *cos* with respect to *P* on the chain polarity of splices for the RecBCD and Rec BC(D$^-$) pathways. The results, shown in Table 8, are similar to those for Red and RecF. For RecBCD we observed 11 *P80*/and 2 *P3*/+ heteroduplexes in cross I and 20 *P3*/+ and 4 *P80*/+ heterduplexes in cross II. The RecBC(D$^-$) crosses showed 13 *P80*/+ and 1 *P3*/+

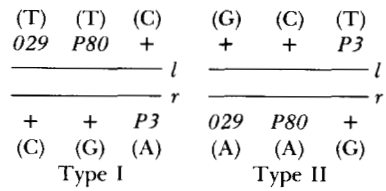
heteroduplex in cross I and 10 *P3*/+ and 4 *P80*/+ heteroduplexes in cross II.

DISCUSSION

Our experiments have focussed on the structure of recombinants heteroduplex at the *P* locus and crossed over for flanking markers (splices). By definition, the structure for a heteroduplex splice is that formed by the joining together of the right arm from one parent to the left arm from the other parent, the junction being formed by overlap of complementary single chain overhangs of the same chemical polarity, each derived from a different parental molecule. We find that for both RecBCD and RecBC(D$^-$), most heteroduplexes are consistent with an overlap of 3′ complementary ends. This holds for crosses with standard *cos* and for crosses with inverted *cos* (*cos*ML).

For the RecF pathway the results of our experiments support the notion of an intermediate having a 3′ overhang sensitive to ExoI. In the light of the previously demonstrated DCB activity of the Red and RecF pathways, these experiments strengthen the evidence in favor of the Red model (STAHL, KOBAYASHI and STAHL 1985) as a description of recombination events for Red and RecF. Strand annealing for Red is proposed to be accomplished by the Redβ protein either acting alone or as a complex with exonuclease (CARTER and RADDING 1971); for RecF, RecA protein promotes strand annealing and exchange. Under certain conditions RecA requires 3′ ends in order to form stable joint molecules (KONFORTI and DAVIS 1987 but see DASGUPTA *et al.* 1981; WU *et al.* 1982). For the Red pathway acting alone or in conjunction with RecA, the proposed 3′ end is considered to be generated by 5′ → 3′ digestion by Red exonuclease. How this may be accomplished for the RecF pathway is less clear. The *recJ* gene is essential for recombination by the RecF pathway (LOVETT and CLARK 1984; KOLODNER, FISHEL and HOWARD 1985; THALER *et al.* 1989). Its gene product has 5′ → 3′ exonuclease activity specific for single stranded overhangs (LOVETT and KOLODNER 1989). It is possible that other proteins such as a helicase or single strand binding protein may act in conjunction with RecJ protein *in vivo* to produce 3′ overhangs from a double chain end.

In the set of crosses involving wild type mismatch repair there is a lack of *P80*/+ heteroduplexes and a distinct disparity in the bias for the two crosses: the bias is considerably greater in cross I (15 *O29*/+ : 1 *P3*/+) than in cross II (17 *P3*/+ : 12 *O29*/+). This may be rationalized in terms of the action of the VSP repair system on the two heteroduplex types:



The VSP system acts to remove the mutant *Pam3* and *Pam80* alleles from the *l* chain. This, in combination with the selection for O^+P^+ information on the *r* chain, frequently removes all mutant alleles from a type II heteroduplex while leaving *Oam29* on the *l* chain of a type I heteroduplex. *P80*/+ is never seen, perhaps because it is obligatorily corrected in type II and very efficiently corrected in type I heteroduplexes.

The experiments involving the RecBCD pathway were done with phage lacking χ. It has been previously proposed that in the absence of χ RecBCD recombination of λ is due to the presence of weak χ-like sequences (CHENG and SMITH 1984). This suggests that under χ^0 conditions recombination proceeds by the same mechanism as when it is stimulated by χ. STAHL *et al.* (1982) argued that χ stimulates splices and patches by the same factor, and ROSENBERG (1987) concluded that chain bias of patches in the RecBCD pathway is the same in the presence as in the absence of χ. There is no evidence we are aware of contradicting the view that χ^+ and χ^0 recombination proceed by the same mechanism. With this in mind we first compare our results with the prediction of the nick-initiation model of SMITH *et al.* (1984), encountering paradoxes. We then suggest the broad outlines of a resolution in terms of a recent model (STAHL *et al.* 1990b) that calls for two distinctly different modes of RecBCD recombination.

Our observations are consistent with the model of SMITH *et al.* (1984), which predicts 3′ splices. The minority class of splices in our experiments may be explained according to this model by branch migration back, past the nicks marking the initial site of invasion. Branch migration past the nicks can occur if they have become ligated. Resolution of the junction in the crossover sense then gives 5′ splices. Within the context of the model our results suggest that branch migration in the reverse direction occurs infrequently or that the nicks tend not to be ligated (see also CHENG and SMITH 1989).

An economical feature of the model is that it accounts for the formation of both crossover and noncrossover products by alternate modes of resolution of a common Holliday junction intermediate. χ does stimulate both such structures ("splices" and "patches," respectively) to the same extent (STAHL *et al.* 1982), and any model that seeks to explain χ action ought to address both. The model predicts that patches arise by single-strand insertions on the *l* chain. ROSENBERG (1987) addressed this prediction and

found heteroduplex patches with recombinant information predominantly on the *r* chain. This is an observation that the simple model of SMITH *et al.* (1984) cannot easily accommodate.

An alternate version of SMITH model was suggested (SMITH *et al.* 1984; ROSENBERG 1987) that can account for *r* chain patches. In this version, the 3′ end invades to form heteroduplex DNA, displacing a D-loop that pairs with its complementary chain in the χ containing parent. The 3′ end then rejoins with the 5′ end at the site of the nick, leading to the formation of a double Holliday junction intermediate. Resolution of both junctions in the vertical sense gives patches on the *r* chain. 3′ splices result from horizontal resolution of the junction proximal to the nick and vertical resolution of the second junction. ROSENBERG (1988) later reported that inversion of *cos* did not change the chain bias–patches were still predominantly on the *r* chain. ROSENBERG pointed out that this result was awkward for all nick-at-Chi models.

The common splice polarity observed for the different pathways is also interpretable in terms of a common (or similar) intermediate for the pathways. From the present understanding of Red and RecF it is conceivable that a 3′ ending single-stranded tail plays a role in all of the pathways examined. The arguments for such a tail in the Red, RecE, and RecF pathways are in INTRODUCTION. THALER *et al.* (1989) proposed that the interaction of RecBCD with χ leads to loss of the D subunit resulting in loss of ExoV activity. In a specific version of that proposal, after the D subunit has been lost at χ, continued unwinding of the DNA by the RecBC(D^-) enzyme (PALAS and KUSHNER 1990) could generate a 3′ ended single-stranded tail which would persist due to absence of ExoV activity and thereby serve as a substrate for recombination.

The chain polarities of RecBCD-mediated patches (ROSENBERG 1987, 1988) on the one hand and splices on the other (this paper) are not easily reconciled. The degrees of freedom required for such a reconciliation may be implicit in the results of STAHL *et al.* 1990b). Those authors argued for two distinct mechanisms of RecBCD-mediated recombination. Their argument was based partly on crosses in which one parent carried χ and the other did not. The recombinant that fails to inherit χ appears to arise via the interaction of one parental phage of each genotype. The recombinant that inherits χ, on the other hand, appears to require for its formation the involvement of one of the χ^0 parent phages and two of the χ^+ ones. A rationalization of this kinetic property of RecBCD recombination proposes that the chromosome that initiates recombination by opening at *cos* is digested from *cos* to χ, at which point ExoV activity is suppressed by loss of the RecD subunit from the RecBCD enzyme. The enzyme continues to travel until it effects reciprocal recombination between two homologously paired chromosomes. The duplex end that was translated from *cos* to χ by the action of ExoV is highly recombinogenic (because of unequal degrees of digestion on the complementary chains and/or the unwinding activity of RecBCD) and invades a third chromosome. This invasion results in "nonreciprocal (or half) crossing over."

The two kinds of recombination postulated by STAHL *et al.* might contribute differently to patch and splice heteroduplexes. For instance, patch heteroduplexes might result primarily from the postulated reciprocal exchanges, while splice heteroduplexes might arise primarily during the nonreciprocal exchanges. Further studies, including ones that measure the lengths of heteroduplexes arising during the two postulated events, might effect the desired reconciliation.

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