EFFECTS OF NONHOMOLOGY ON BACTERIOPHAGE LAMBDA RECOMBINATION

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ABSTRACT

When crosses are performed under conditions severely restricting DNA synthesis, the presence of DNA sequence nonhomologies in the *lac* region of λ*plac*5 limits the parental material contribution to and the yield of phage recombinant in a region bracketed by the nonhomologies. These observations are consistent with the expectation of a role for branch migration in the formation of heteroduplex structures under these conditions. Under conditions permissive for DNA replication, bracketing a region with nonhomologies has an only modest effect on the yield of recombinants within that interval. In addition, recombinants within such a bracketed interval manifest an excess of coincident exchange events in an adjacent region. These observations suggest the possibility that, under conditions permissive for DNA replication, regions of nonhomology can be included in heteroduplex structures.

SEVERAL lines of investigation have provided a basis for thinking about generalized λ recombination as a process that involves the formation of heteroduplex overlap structures in the region where parental DNA molecules are joined (for review, see FOX 1978). Heterozygous phage are observed among the progeny emerging from a λ cross, especially among the unduplicated products of such a cross (MESELSON 1967a), and the progeny phage which have enjoyed recombination between two closely linked markers often are heterozygous for nearby markers (AMATI and MESELSON 1965). In addition, phage emerging from λ crosses often appear to have enjoyed clusters of multiple genetic exchanges in regions defined by closely linked genetic markers (AMATI and MESELSON 1965). One explanation for this phenomenon, known as localized negative interference, is that the apparent multiple crossovers are the products of a single molecular exchange event which included some, if not all, of the markers in question in a region of heteroduplex DNA. Subsequent processing by mismatch repair could give rise to products that manifest several genetic exchanges.

Proposals describing the mechanism of recombination which include as an intermediate step the formation of heteroduplex DNA have proliferated (for review, see HOTCHKISS 1974b). One such model, proposed by HOLLIDAY (1964) to account for certain features of fungal recombination, has of late found increasing acceptance as a description of the molecular events that occur in bacterial and bacteriophage recombination. This model incorporates as an early step in

the recombination process the formation of a two-strand crossover structure in which the two parental homologs are linked by an exchange of equivalent DNA strands. Breakage and reformation of equivalent base pairs in the two duplex structures would allow formation of heteroduplex DNA by translocation of the two-strand crossover structure. Both theoretical (MESELSON 1972; SIGAL and ALBERTS 1972) and direct experimental (THOMPSON, CAMIEN and WARNER 1976; WARNER, FISHEL AND WHEELER 1978) examination of this translocation process (termed branch migration) have suggested that it can occur without any substantial change of free energy due to loss of base pairing. It might be expected that the presence of a substantial sequence nonhomology would constitute a barrier to branch migration, since inclusion of a nonhomology in heteroduplex DNA would involve formation of considerable sequences of unpaired bases.

Evidence that supports the HOLLIDAY model as a reasonable description of some of the features of λ recombination has come from studies that examined the recombinant progeny that emerge from replication-restricted λ crosses. In these crosses, DNA synthesis was severely restricted by virtue of mutations in both the infecting phage (O^- or P^-) and in the bacterial host (*dnaB*$^-$). Since phage emerging from these replication-restricted crosses are the unduplicated products of recombination (MCMILIN and RUSSO 1972; STAHL *et al.* 1973), an assessment of the nature of the parental material contribution to joint molecules can be made by labeling one of the infecting parents with heavy isotopes and by displaying the emerging progeny phage on an equilibrium density gradient. WHITE and FOX (1974) utilized this methodology to examine recombination which occurs in the *OP* region of λ. Phage emerging from replication-restricted crosses which were recombinant in this short genetic interval appear to derive predominantly from crossover products in which the entire interval was present as heteroduplex DNA (see also STAHL and STAHL 1974). SODERGREN and FOX (1979) utilized the same methodology to examine the effects of nonhomologies on recombination in replication-restricted crosses, specifically, on recombination in the *lac* region of λ*plac5*. When both infecting parents harbored *lacZ*$^-$ point mutations, *lacZ*$^+$ recombinants exhibited a broad range of parental DNA compositions, consistent with their arising from phage that contained large stretches of heteroduplex DNA including the *lac* region. When either one or both parents harbored *lacZ*$^-$ deletion or insertion mutations, the parental contribution to, and the yield of, *lacZ*$^+$ recombinant progeny was limited in a manner that suggested that the nonhomology mutations were excluded from regions of heteroduplex DNA. These results led SODERGREN and FOX to conclude that, under replication-restrictive conditions, recombinant phage were generated by crossover events that formed heteroduplex DNA in a manner consistent with the mechanism of branch migration within a structure of the kind proposed in the HOLLIDAY model.

It was of interest to determine whether the recombinant phage which emerge from normal lytic λ crosses are generated by a similar mechanism. An examination of the direct products of exchange events in these crosses is confounded by the fact that, in lytic infections, replication and recombination are occurring at the same time, and most of the recombinant phage emerging from such

crosses have enjoyed a substantial amount of replication (MESELSON and WEIGLE 1961; MESELSON 1967b). We have examined the effect of substantial sequence nonhomologies on the yield of phage that have enjoyed both single and multiple exchanges in or near the *lac* region of λ*plac5*. We have found that, although some of the crossovers that occur in vegetative λ crosses exhibit properties consistent with their derivation from a HOLLIDAY-type intermediate, a substantial fraction of the recombination events must occur via a mechanism that is insensitive to the presence of sequence nonhomologies.

MATERIALS AND METHODS

Bacterial strains: Bacterial strains are listed and described in Table 1. Strains listed without a source or a reference were constructed for this study. All strains are derivatives of *E. coli* K12.

Phage strains: All phage strains used in this work are derivatives of λ*imm434 plac5*, a gift of L. SOLL. Appropriate genetic markers (*imm*λ, *Pam3*, *Pam80*, *Jam449*, *red3* and various *lacZ* mutations) were crossed onto λ*imm434* by conventional crosses with the phage, or derivatives of the phage, listed in Table 2. λ*imm434 plac5 lacZamYA482*, λ*imm434 plac5 lacZamNG200* and λ*imm434 plac5 lacZocAP2202* were obtained from *lacZ*$^-$ homogenotes of λ*imm434 plac5* lysogens of the appropriate bacterial strain.

λ*imm434 plac5* derivatives carrying the double mutations *lacZamNG200 lacZMS348* or *lacZocAP2202 lacZΔW4680* were obtained from conventional phage crosses between λ*plac5* derivatives carrying each single marker. Presence of the nonsense alleles was confirmed by recovery of those alleles from crosses between λ*plac5* derivatives containing the *lacZ*$^-$ double mutations and λ*plac5* derivatives containing a wild-type *lacZ* gene. Presence of the deletion and insertion mutations was confirmed by restriction mapping.

A map of the relevant λ genes, and of the *lacZ* mutations used as genetic markers in the study, is given in Figure 1.

Media and buffers: Bacteria on which phage were plated were grown in λ broth (WHITE and FOX 1974) supplemented with 0.01 (w/v) Difco Bacto yeast extract and 0.2% (w/v) maltose. Bacteria in which crosses were carried out were grown in that medium containing 0.01 M $MgSO_4$. Phage were plated on BBL trypticase agar plates (WHITE and FOX 1974) or on lac-tetrazolium agar plates (λ agar (SIGNER and WEIL 1968) supplemented with 0.5% (w/v) lactose and 0.05% 2,3,5-triphenyltetrazolium chloride (Sigma Chemical Co.) after autoclaving). In all cases, λ top agar (SODERGREN and FOX 1979) was used. Phage were diluted and stored in either SM buffer (WHITE and FOX 1974) or in supplemented λ broth containing $MgSO_4$.

Phage stocks: Phage stocks were prepared by the plate lysate method.

Replication-restricted phage crosses: Bacterial strain FA77 was grown in supplemented λ broth containing $MgSO_4$ at 26° to a concentration of 2×10^8 cells/ml. Parental phage were mixed at equal concentrations in supplemented λ broth containing $MgSO_4$ to a final concentration of 2 to 4×10^9 phage/ml each. Bacteria and phage mixes were aerated separately for 15 min at 42°, mixed in equal volumes, and allowed to adsorb at 42° for 15 min without agitation. Aeration was then resumed and allowed to continue for 90 min. The cross mixtures were then diluted 10^{-2} into SM buffer, and a few drops of chloroform were added. The lysate was then diluted and titered on the appropriate bacterial indicators.

Replication-permitted phage crosses: The bacterial host was grown at 37° in supplemented λ broth containing $MgSO_4$ to a concentration of 2×10^8 cells/ml. Equal volumes of cells and of a phage mix containing 1×10^9 phage/ml of each parental phage was mixed, and phage were allowed to adsorb without agitation for 15–20 min at 37°. This procedure generally resulted in greater than 90% adsorption of the input phage. The adsorption mix was diluted 10^{-2} to 10^{-4} into prewarmed supplemented λ broth containing $MgSO_4$. After 1.5 to 2 hr of aeration at 37°, a few drops of chloroform were added to lyse the cells, and the lysate was diluted and titered on the appropriate indicator strains.

Since the *lacZ* gene contains a *chi* site (TRIMAN, CHATTORAJ and SMITH 1982), all replication-permitted crosses were performed in *recB*$^-$ hosts, where *chi* is not active (STAHL and STAHL 1977).

TABLE 1

Bacterial strains

Strain designation	Relevant genotype	Source or reference
FA77	*dnaBts* Su^-	McMilin and Russo (1972)
XA102	*supE*$^-$ Δ*(lacpro)X111*	J. Miller via D. Botstein
XA10B	*supB*$^-$ Δ*(lacpro)X111*	J. Miller via D. Botstein
MJL41	*recB21* derivative of XA102	
MJL171	*recA1 recB21* derivative of XA102	
JC7623	*recB21 recC22 sbcB15 supE*$^-$	A. Clark via G. Walker
MJL201	*lacZΔU169* derivative of JC7623	
M182	*Su*$^-$ *lacZΔX74*	J. Beckwith
MJL12	λ*imm434 c17 Oam29* lysogen of M182	
MJL80	λ*c17 Oam29* lysogen of M182	
MJL92	λ*imm434 c17 Oam29 Jam449* lysogen of M182	
MJL147	λ*c17 Oam29 Jam449* lysogen of M182	
MJL162	λ*imm434 c17 Oam29 Pam80* lysogen of M182	
NG200	*Su*$^-$ *lacZamNG200*	Morrison and Zipser (1970)
MJL156	λ*imm434 c17 Oam29 Jam449* lysogen of NG200	
MJL157	λ*c17 Oam29 Jam449* lysogen of NG200	
AP2202	*Su*$^-$ *lacZocAP2202*	Morrison and Zipser (1970)
M7023	*Su*$^-$ *lacZamYA482*	J. Beckwith
M5146	*Su*$^-$ *lacZΔr1*	E. Signer via D. Botstein

TABLE 2

Primary sources of phage genetic markers

Phage strains	Source
λ*cI857*	D. Botstein
λ*Jam449*	E. Signer
λ*c60 Pam3*	E. Signer
λ*Pam80*	E. Signer
λ*red3*	E. Signer
λ*cI857 Sam7 plac5 lacZΔM15*	M. H. Malamy
λ*cI857 Sam7 plac5 lacZΔW4680*	M. H. Malamy
λ*cI857 Sam7 plac5 lacZMS520*	M. H. Malamy
λ*cI857 Sam7 plac5 lacZMS505*	M. H. Malamy
λ*cI857 Sam7 plac5 lacZMS319*	M. H. Malamy
λ*cI857 plac5 lacZMS348*	M. H. Malamy
λ*imm434 plac5 lacZamYA482*	This work
λ*imm434 plac5 lacZamNG200*	This work
λ*imm434 plac5 lacZocAP2202*	This work
λ*imm434 plac5 lacZamNG200 lacZMS348*	This work
λ*imm434 plac5 lacZocAP2202 lacZΔW4680*	This work

Titration methodology—two-factor crosses: In the two-factor crosses reported in Tables 4 and 5, total phage were titered on BBL trypticase agar plates, using a lawn of MJL12. *lacZ*$^+$ phage were titered on lac-tetrazolium agar plates, using the same indicator strain. *lacZ*$^+$ phage form dark red plaques on these plates and could be easily and reproducibly detected among a background of 2×10^5 *lacZ*$^-$ plaques.

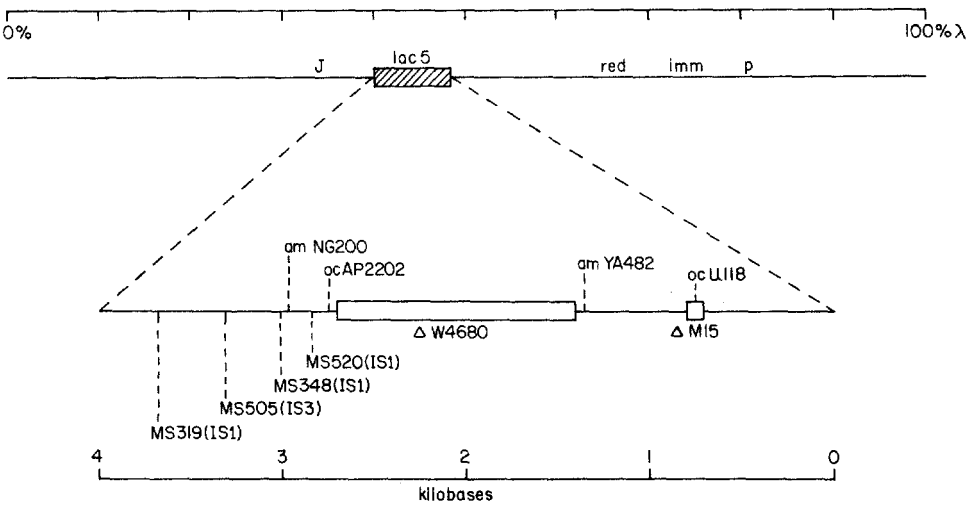


FIGURE 1.—A physical map of λ*plac*5. The position of various genes and of the *lac*5 and *imm434* substitutions are taken from DAVIS, BOTSTEIN and ROTH (1980). The positions in the *lac* region of λ*plac*5 (expanded map) of the insertion mutations and of Δ*W4680* are taken from MALAMY, FIANDT and SZYBALSKI (1972). The position of Δ*M15* was derived from published DNA and protein sequences of *lacZ* (BUCHEL, GRONENBORN and MÜLLER-HILL 1980; FOWLER and ZABIN 1977) and from restriction mapping studies (M. LICHTEN and M. S. FOX, unpublished results). The relative positions of *amNG200*, *ocAP2202*, and *amYA482* were determined by deletion mapping, as described by SODERGREN and FOX (1979), and by multifactor crosses (LICHTEN and FOX, unpublished results). Approximate positions of point mutations were determined by two-factor crosses (data not shown).

Titration methodology—multifactor crosses: In the multifactor crosses reported in Table 6, total phage were titered on BBL trypticase agar plates, using lawns of MJL12 and MJL80 as appropriate. *lacZ*$^+$ phage were titered on lac-tetrazolium agar plates, using the same bacterial lawns. *lacZ*$^+$ *J*$^+$ phage were titered on lac-tetrazolium agar plates, using lawns of MJL92 and MJL147 or M182, as appropriate. *imm*λ *P*$^+$ and *imm*λ *P*$^+$ *lacZ*$^+$ phage were titered on BBL trypticase agar plates and lac-tetrazolium agar plate, respectively, using a lawn of MJL162.

In multifactor crosses in which *lacZamNG200* was used as a central marker, *J*$^+$ phage containing the wild-type allele of *lacZamNG200* were titered on lac-tetrazolium agar plates, using a lawn of MJL156 or MJL157, as appropriate. *lacZ*$^+$ phage and phage carrying only the *lacZΔM15* or *lacZΔW4680* alleles form red plaques on these indicators, presumably due to a combination of recombination with, and intracistronic complementation by, the resident *lacZamNG200* gene on the bacterial chromosome (LANGLEY *et al.* 1975). Similarly, in crosses in which *lacZamYA482* or *lacZΔW4680* were used as a central marker, *J*$^+$ phage carrying the wild-type allele of these mutations and either the wild-type or mutant allele of *lacZΔM15* were titered on lac-tetrazolium agar plates, using M5146 as an indicator. In all of these instances, *J*$^+$ phage carrying the wild-type allele for these central markers could be detected easily and reproducibly among a background of 2×10^4 phage/plate. A summary of the plating characteristics of the strains described will be found in Table 3.

Titration methodology—general: In all crosses, plates were incubated at 34° for 15–24 hr, counted, and then incubated at room temperature for an additional 24 hr. The plates were then recounted in order to detect late-arising red plaques, even though these were encountered infrequently. A minimum of 50–100 plaques were counted on each indicator. To keep the number of total phage per plate as low as possible, a large number of plates were used when recombination frequencies were low. Appropriate reconstruction experiments and controls for recombination on the plates were performed. The methods outlined were found to be quantitative, and no significant contribution arising from plate-recombination was observed.

TABLE 3

Characteristics of indicator strains

Strain name	Plates phage carrying			Positive indicator for phage carrying *lacZ* alleles
	imm allele	*P* alleles	*J* alleles	
M182	*434*, λ	+	+	+
MJL12	λ	+ or *am*	+ or *am*	+
MJL80	*434*	+ or *am*	+ or *am*	+
MJL92	λ	+ or *am*	+	+
MJL147	*434*	+ or *am*	+	+
MJL162	λ	+	+ or *am*	+
NG200	*434*, λ	+	+	+, Δ*M15*, Δ*W4680*
MJL156	λ	+ or *am*	+	+, Δ*M15*, Δ*W4680*
MJL157	*434*	+ or *am*	+	+, Δ*M15*, Δ*W4680*
M5146	*434*, λ	+	+	+, Δ*M15*

RESULTS

Experimental design of two-factor crosses: If recombinants are generated by a mechanism that forms stretches of heteroduplex DNA via branch migration, then most of the phage that enjoy recombination in a genetic interval which is shorter than the average length of heteroduplex should be generated by crossover events that initiated outside the interval and subsequently migrated into or through that interval. If significant sequence nonhomologies constitute a barrier to such branch migration, then it might be expected that the close bracketing of a short genetic interval would significantly reduce the frequency of recombination in that interval, since the interval would be closed to the entry of branch-migrating crossover structures.

An experiment that tests this prediction is outlined schematically in Figure 2. The frequency of recombination in a genetic interval (in this case, the region between a point mutation and an insertion or deletion mutation) is measured in two different crosses. In the first cross (point × nonhomology), the interval is OPEN on one side to branch migration, and recombinants can be generated either by crossover events which occur within the interval or by crossover events which initiate outside the interval and subsequently branch migrate into it. In the second cross (nonhomology-point double mutant × nonhomology), the interval is bracketed by nonhomologies. If extension of heteroduplex is prominently blocked by the presence of nonhomologies, the interval would be CLOSED to entry by crossover structures that initiated outside the interval. Recombinants would be generated only by crossover events that initiated within the interval itself. By comparing the yield of recombinants from crosses in which an interval is in either the OPEN or CLOSED configuration, one can estimate the relative contribution of outside- and inside-initiating crossover events to recombination in a given genetic interval.

Results of replication-restricted two-factor crosses: Phage emerging from replication-restricted crosses have been shown to contain extensive regions of heteroduplex DNA (FOX, DUDNEY and SODERGREN 1979), and significant se-

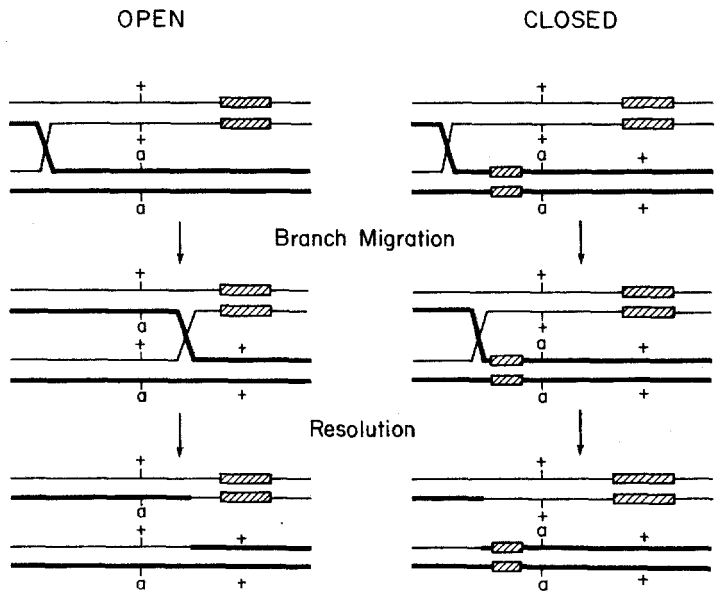


FIGURE 2.—Design of two-factor crosses. If a genetic interval is bounded on one side by a point mutation (OPEN configuration), branch-migrating crossover structures can enter the interval and can resolve in such a way that a wild-type recombinant is formed. If a genetic interval is bracketed by nonhomologies (CLOSED configuration), branch-migrating structures cannot enter the interval and, thus, cannot resolve to form wild-type recombinants.

quence nonhomologies appear to constitute a barrier to the formation of that heteroduplex (SODERGREN and FOX 1979). It was, therefore, of interest to determine whether the predictions outlined in the preceding section would hold. The effect of bracketing by nonhomologies on the frequency of recombination in a series of genetic intervals of increasing length in the *lacZ* gene of λ*plac5* was examined in crosses performed at 42° in the Su^-, *dnaBts* host FA77, using parental phage that contained nonsense mutations in *P*. Under these conditions, DNA synthesis is limited by mutations in the host ($dnaB^-$) and in the input phage (P^-). The results of these crosses are presented in Table 4. Bracketing a region as large as 2200 nucleotides in length has the effect of greatly reducing the yield of recombinants in that interval. This reduction depends upon the size of the interval and ranges from 20- to 54-fold. In the crosses presented in the first part of Table 4, both the *rec*-dependent recombination system of *E. coli* and the *red*-dependent recombination system of λ were functional. Qualitatively, similar results were obtained when the input phage were exo^- bet^- by virtue of the *red3* mutation (second part of Table 4), indicating that the results presented previously reflect properties of the *rec*-dependent system of recombination.

Results of replication-permitted two-factor crosses: Because the experimental approach outlined previously is purely genetic in nature, it can be used to study the mechanism of recombination in λ crosses performed under conditions in which DNA synthesis is unrestricted and analysis of the direct products of a

TABLE 4

Results of replication-restricted two-factor crosses

lacZ markers			OPEN	CLOSED	
a	*b*	Distance[a]	f(*lac*$^+$)[b] × 10^4	f(*lac*$^+$)[b] × 10^4	f(*lac*$^+$) OPEN / f(*lac*$^+$) CLOSED
red$^+$ *crosses*					
NG200	*MS520*	0.17	47	0.9	54
NG200	*ΔW4680*	0.31	64	2.5	26
NG200	*ΔM15*	2.2	160, 120	6.1, 5.9	26, 20[c]
red$^-$ *crosses*					
NG200	*MS520*	0.17	92	1.9	48
NG200	*ΔW4680*	0.31	110	3.9	28
NG200	*ΔM15*	2.2	160	11	15

All crosses were performed in the nonpermissive host FA77 (*dnaBts*, *Su*$^-$) at 42°, as described in MATERIAL AND METHODS. Parental phage were either *cI857 Pam80* or *Pam3* derivatives of λ*plac5* and were either *red*$^+$ or *red3* as indicated. In the crosses labeled OPEN, one parent phage contained as marker *a* *lacZamNG200*. In the crosses labeled CLOSED, one parent phage contained as marker *a* the double mutation *lacZamNG200 lacZMS348*. In both crosses, the other parent phage contained as marker *b* the insertion or deletion mutations indicated.

[a] Distance, in kilobases, between the two bracketing nonhomologies.

[b] *lac*$^+$ phage per total phage.

[c] Results of two separate crosses.

recombination event is not possible. A series of two-factor crosses were carried out in the permissive host MJL41 (*recB*$^-$, Su$^+$), using *red*$^+$ parental phage. The results of these crosses are presented in the first part of Table 5. Under these conditions (*red*$^+$, replication$^+$) the effect of bracketing an interval on recombination in that interval is much more modest than that observed in replication-restricted crosses—a three- to four-fold reduction if the interval is about 150 nucleotides in length, a two- to three-fold reduction if the interval is about 300 nucleotides in length, and no reduction in recombination frequencies if the interval is 1000 nucleotides or greater in length. These results reflect the effect of nonhomologies on crossover events promoted by the products of the *red* genes (exo and *bet*) of λ, as quantitatively similar results were obtained from crosses performed in the *recBC*$^-$ *sbcB*$^-$ host MJL201 and from crosses performed in the *recA*$^-$*recB*$^-$ host MJL171 (data not shown).

A similar series of crosses was performed, using parental phage carrying the *red3* mutation, in the *recBC*$^-$ *sbcB*$^-$ host MJL201, in which the *recF* pathway of recombination is fully active (CLARK 1974). Results of these crosses are presented in the second part of Table 5. Here, the effect of bracketing nonhomologies on recombination in an interval appears to be intermediate between that observed in replication restricted crosses and that observed in *red*$^+$, replication-permitted crosses. Bracketing an interval 2200 nucleotides in length reduces recombination in that interval twofold, whereas bracketing an interval about 150 nucleotides in length causes an almost tenfold reduction in recombination in that interval. Qualitatively, similar results were observed when *red*$^-$ crosses were performed in the *recB*$^-$ host MJL41, in which the *recF* pathway of recombination should be active at a reduced level (CLARK 1974). However, the yield of recombinants from these crosses was so low (30- to 50-fold reduced relative to *red*$^+$ crosses in the

TABLE 5

Results of replication-permitted two-factor crosses

lacZ markers			OPEN	CLOSED	f(*lac*$^+$) OPEN
a	b	Distance[a]	f(*lac*$^+$)[b] × 10^4	f(*lac*$^+$)[b] × 10^4	f(*lac*$^+$) CLOSED
red$^+$ crosses					
AP2202	*MS520*	0.14	3.7	1.1	3.4
NG200	*MS520*	0.17	5.0, 3.8	1.2, 1.1	4.2, 3.5[c]
NG200	*ΔW4680*	0.31	6.9, 5.5	2.2, 3.0	3.1, 1.8[c]
AP2202	*MS348*	0.31	9.6	5.4	1.8
AP2202	*MS505*	0.64	16	11	1.5
AP2202	*MS319*	0.97	20	18	1.1
NG200	*ΔM15*	2.2	33, 45	41, 48	0.8, 0.9[c]
red$^-$ crosses					
NG200	*MS520*	0.17	0.96	0.10	9.6
NG200	*ΔW4680*	0.31	1.7	0.26	6.5
NG200	*ΔM15*	2.2	20	10	2.0

red$^+$ crosses were performed in the permissive host MJL41 (*recB*$^-$, *supE*$^-$), and *red*$^-$ crosses were performed in the permissive host MJL201 (*recBC*$^-$*sbcB*$^-$, *supE*$^-$) as described in MATERIALS AND METHODS. Parental phage were either *cI857 Pam80* or *Pam3* derivatives of λ*plac5* and were either *red*$^+$ or *red3* as indicated. In crosses labeled OPEN, one parent carried as marker a either *lacZamNG200* or *lacZocAP2202*; in the corresponding crosses labeled CLOSED, marker *a* was either the double mutation *lacZamNG200 lacZMS348* or the double mutation *lacZocAP2202 lacZΔW4680*. In all crosses, the second parent carried as marker *b* the insertion or deletion mutation indicated.

[a] Distance, in kilobases, between the two bracketing nonhomologies.

[b] *lac*$^+$ phage per total phage.

[c] Results of two separate crosses.

same host) that accurate recombination frequencies could not be determined. For this reason, data from these crosses are not reported.

Experimental design of multifactor crosses: When three-factor λ crosses involving closely linked markers are carried out, the yield of double recombinants observed exceeds that predicted on the basis of the frequency of the two independent single crossover events (AMATI and MESELSON 1965). One way of accounting for this phenomenon (termed localized negative interference) is to suggest that at least the central of the three markers is included in a region of heteroduplex DNA. In addition, apparent multiple crossovers could be generated by subsequent processing of the product of a single exchange event that contained more than one of the three markers in heteroduplex DNA. The work of WHITE and FOX (1974) on recombination in the right arm of λ in replication-restricted crosses has lent support to this notion. Phage that gave rise to multiple recombinants in the *OP* region exhibited allelic compositions and parental material contributions consistent with the frequent inclusion of the entire *OP* region included in heteroduplex DNA.

Examination of the phenomenon of localized negative interference might provide an additional test of the sensitivity of various recombination systems to nonhomologies. If most of the double recombinants emerging from a three-factor cross are generated by inclusion of at least the central marker in heteroduplex, and if nonhomologies are not included in heteroduplex DNA, then no localized negative interference should be observed in three-factor

crosses in which the central marker is either an insertion or deletion mutation. However, if recombination occurs by a process that is able to include nonhomologies in heteroduplex, excess double recombinants would still be observed. A schematic representation of such an experiment is presented in Figure 3.

Results of multifactor crosses: This hypothesis has been tested in a series of three-factor crosses in which the central marker was a point mutation, a deletion mutation or an insertion-point double mutation. Two of the three markers were located in the *lacZ* gene of λ*plac5*, and the third was located in the *J* gene adjacent to *lacZ*. The fraction of doubly recombinant $lacZ^+$ J^+ phage was determined. Since negative interference can also be caused by probabilistic effects such as unequal multiplicities of infection or nonrandom intracellular mating (VISCONTI and DELBRÜCK 1953), we also determined the frequency of double recombinant progeny that were recombinant in *lacZ* and in the region between immunity and *P*, about 15,000 nucleotides away. The results of these multifactor crosses, carried out with red^+ parental phage in the permissive host MJL41 ($recB^-$, Su^+), are displayed in the first part of Table 6.

In all the red^+ crosses in which it was measured, global negative interference (i_{bc}) was observed to have a value not far from unity, indicating that, under these conditions, mating is nearly random. In contrast, excess double recombinants were observed in adjacent intervals in the center of the chromosome (i_{ab} ranges from 5.5 to 12.6), even when the central marker contained either a

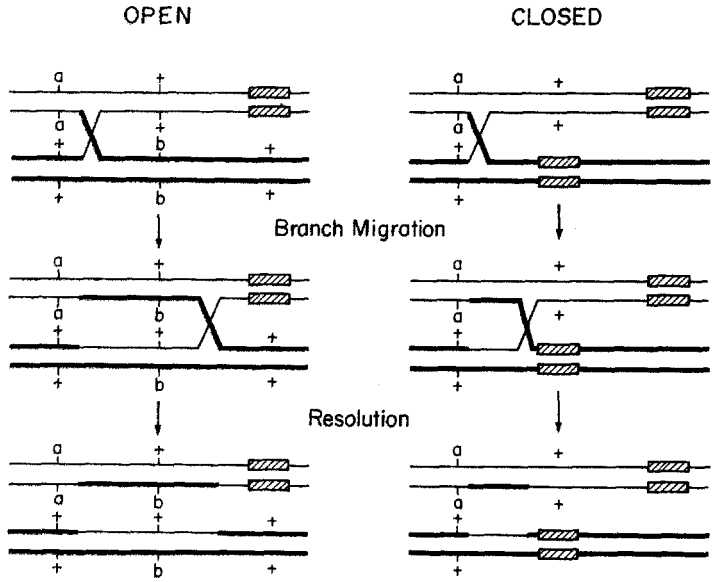
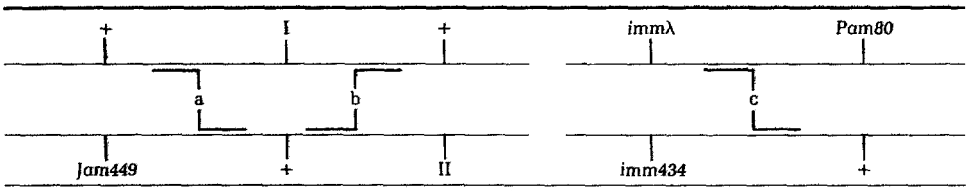


FIGURE 3.—Design of three-factor crosses. If the marker shared by two adjacent genetic intervals is a point mutation (OPEN configuration), HOLLIDAY-type crossover structures can branch migrate from the first interval into the second interval and can resolve in such a way that a double recombinant is formed. If the central marker includes a nonhomology, in this case a deletion mutation (CLOSED configuration), HOLLIDAY-type crossover structures cannot migrate from the first interval to the second and, thus, cannot resolve to form a double recombinant.

TABLE 6

Results of multifactor crosses



Markers in *lacZ* I	II	f(a) × 10^3	f(b) × 10^4	f(ab)/f(b)	i_{ab}[a]	f(c) × 10^2	f(bc) × 10^5	i_{bc}[a]
red$^+$ crosses								
NG200	*ΔM15*	9.1	48	0.15	8.1	1.6	27	1.8
NG200MS348	*ΔM15*	12	47	0.13	5.6	1.8	25	1.5
NG200	*ΔW4680*	9.6	9.3	0.18	9.5	1.4	3.8	1.6
NG200MS348	*ΔW4680*	10	4.2	0.11	5.5	1.6	2.2	1.6
NG200	*MS520*	n.d.	5.2	0.25	12.5[b]	1.6	2.1	1.6
NG200MS348	*MS520*	n.d.	1.5	0.11	6.1[b]	1.6	0.45	0.94
YA482	*ΔM15*	13	18	0.24	9.4	n.d.	n.d.	n.d.
ΔW4680	*ΔM15*	6.6	14	0.11	8.7	n.d.	n.d.	n.d.
red$^-$ crosses								
NG200	*ΔM15*	4.9	23	0.20	20	0.35	3.2	2.0
NG200MS348	*ΔM15*	6.7	13	0.067	5.0	0.31	1.9	2.4
YA482	*ΔM15*	4.0	3.7	0.34	42	n.d.	n.d.	n.d.
ΔW4680	*ΔM15*	2.4	2.1	0.05	9.9	n.d.	n.d.	n.d.

red$^+$ crosses were performed in the permissive host MJL41 (*recB*$^-$, *supE*$^+$), and *red*$^-$ crosses were performed in the permissive host MJL201 (*recBC*$^-$*sbcB*$^-$, *supE*$^-$), as described in MATERIALS AND METHODS. Parental phage were *red*$^+$ and *red3* derivatives of λ*plac5*. Other details of parental phage genotypes are provided in the table heading, with the exception that, in crosses in which *lacZ* marker I was either *ocYA482* or *ΔW4680*, both parents were *imm434* P$^+$. f(a), f(b), f(c), f(ab) and f(bc) are the frequencies of single and double crossovers in the intervals and directions indicated in the table heading. n.d. indicates that no determination was made.

[a] i_{ab} = R(ab)/R(a)R(b), where R(x) = the total frequency of crossovers (in both directions) in the interval x. In this work, the total frequency of crossovers in an interval was assumed to be twice the frequency of crossovers in the measured direction. i_{ab} = 1 would indicate that no more double crossovers were observed than might be predicted on the basis of the frequencies of single crossovers observed.

[b] Because of the scoring methods used, f(a) could not be determined in these crosses. Instead, the average f(a) for the other four *red*$^+$ crosses, which were performed at the same time, was used in calculating i_{ab}.

deletion or an insertion mutation. When the central marker was a point mutation, about 20% of the *lacZ*$^+$ were also recombinant in the adjacent interval; when the central marker contained either a deletion or insertion mutation, about 11% of the *lacZ*$^+$ progeny were also *J*$^+$. Quantitatively similar results were observed in *red*$^+$ crosses performed in MJL171 (*recAB*$^-$) and in MJL201 (*recBC*$^-$*sbcB*$^-$), suggesting that the results reflect properties of the *red*-dependent system of λ recombination.

Because the results suggested that, in vegetative λ growth, it was possible that the *red*-dependent recombination system could include nonhomologies in regions of heteroduplex, it was of interest to determine whether the *recF* pathway of recombination had similar properties. Multifactor crosses of the type de-

scribed were performed in the permissive host MJL201 (*recBC*$^-$ *sbcB*$^-$, Su$^+$), using parental phage that carried the *red*3 mutation. The results of these crosses are displayed in the second part of Table 6. Global negative interference was observed to assume a value of about 2. Significant localized negative interference was observed (i_{ab} ranged from 5.0 to 42). When the central marker was a point mutation, 20 to 34% of the *lacZ*$^+$ phage were also *J*$^+$; when the central marker included an insertion or deletion mutation, about 6% of the *lacZ*$^+$ phage were also *J*$^+$.

DISCUSSION

Results of two-factor crosses suggest that a symmetric two-strand exchange structure might play a role in the formation of λ recombinants: A feature of the HOLLIDAY model of recombination that has been the focus here is the mechanism by which regions of heteroduplex DNA are formed. If branch migration occurs by a passive process, with no significant net input of energy, then extension of heteroduplex DNA by a two-strand crossover structure should be blocked by the presence, in one of the exchanging partners, of a substantial sequence nonhomology. If a short genetic interval is bracketed by nonhomologies, it should be closed to the entry of branch-migrating crossover structures, and such entry would no longer contribute to recombination in that interval.

The results of the two-factor crosses presented in this study are, at first examination, consistent with recombination proceeding via a HOLLIDAY-type mechanism in both replication-restricted and replication-permitted crosses. Under all three conditions examined (replication-restricted, *red*$^+$ replication-permitted and *red*$^-$ replication-permitted), the close bracketing of a genetic interval with nonhomologies results in a significant reduction in the yield of phage that have recombined in that interval, if the genetic interval is suitably small. These observations are consistent with the notion that recombination, under all the conditions examined, may proceed via branch migration of a HOLLIDAY-type intermediate, but that the average distance of branch migration differs depending upon the intracellular environment encountered. However, these results do not exclude the possibility that in some fraction of the cases recombination proceeds in a manner that can accommodate the presence of nonhomologies within heteroduplex structures.

Results of multifactor crosses suggest that at least some of the crossovers which occur under freely replicating conditions can include nonhomologies in regions of heteroduplex DNA: The phenomenon of localized negative interference, which refers to the unexpectedly high incidence of multiple genetic exchanges in short genetic intervals, has often been accounted for by suggesting that multiple genetic exchanges in an interval derive from crossover products that include at least part of the interval in heteroduplex DNA. In the multifactor crosses reported in this study, multiple genetic exchanges in two adjacent intervals in the central portion of the λ genome occurred at an unexpectedly high frequency, and excess multiple exchanges were observed when the central marker included either a deletion or an insertion mutation. If the suggested hypothesis is justified, then these results indicate that, in replication-permitted

crosses, recombinants are generated via a mechanism that can at least sometimes accommodate heteroduplex DNA that contains either a deletion or an insertion nonhomology. Both the *red*-dependent recombination system of λ and the *recArecF*-dependent recombination system of *E. coli* appear to be able to form such heteroduplex DNA, albeit to a different extent.

The validity of this interpretation is contingent upon the demonstration that the excess double recombinants observed are not attributable to cell to cell or intracellular heterogeneities in the opportunity for heterozygous exchange (AMATI and MESELSON 1965; VISCONTI and DELBRÜCK 1953). The observation that, in the multifactor crosses reported here, phage that have recombined in *lac* and in the region between *imm* and *P* are present only in modest excess (the average global interference index in red^+ crosses is about 1.5, in red^- crosses, about 2.2) indicates that the substantially greater excess of lac^+I^+ double recombinants observed cannot be entirely due to statistical effects but must at least in part be due to some structural aspect of the recombination event itself. It should be noted that the low global interference indexes observed in these crosses are not due to either the choice of regions of the λ chromosome examined or to the presence of nonhomologies in the parental phage genomes. Similar global interference indexes have been measured in crosses in which all of the markers used were point mutations and in which the genetic intervals examined were located elsewhere on the λ genome (M. LICHTEN and M. S. FOX, unpublished observations). Although other workers (KAISER 1955; AMATI and MESELSON 1965) have reported higher global interference indexes (ranging from about 3 to about 5), these were obtained using an infection procedure that utilized cells grown in the absence of maltose. It is possible that this procedure resulted in a greater variance in parental input multiplicities due to poor adsorption, contributing to the higher global interference indexes observed.

Experiments performed prior to the initiation of the work reported in this paper examined the effect of nonhomologies on recombination in the neighborhood of the immunity region of λ (MAKIN, SZYBALSKI and BLATTNER 1982). High levels of localized negative interference were observed in three-factor crosses in which the central marker included in scored double recombinants was either a deletion or a double point mutation. They reported much lower frequencies of double crossovers when the central marker included in double recombinants was the wild-type allele of a deletion. In the three-factor crosses in the *lac* region of λ*plac5* reported in this paper, the wild-type alleles of both deletion and insertion mutations were included in double recombinants with very nearly equal efficiencies. Although the precise basis of the difference between the observations of MAKIN, SZYBALSKI and BLATTNER (1982) and the results of the multifactor crosses reported in this paper remains to be determined, it may reflect a difference in the nature of recombination events and their relative prominence in different portions of the λ genome. Alternatively, it may reflect the presence or the levels of an activity in the crosses of MAKIN, SZYBALSKI and BLATTNER (1982) which could be responsible for preferential excision of the single strand loop present in heteroduplex molecules hybrid for a nonhomology. This could lead to the preferential loss of the wild-type allele of a deletion

mutation from such heteroduplex structures. BENZ and BERGER (1973) have proposed such an activity to account for the selective loss of the wild-type allele from crosses between wild-type and *rII*-deletion mutant strains of bacteriophage T4.

Other genetic systems displaying evidence for formation of heteroduplex DNA containing nonhomologies: The results of the multifactor crosses reported here and their interpretation might be more surprising had not investigations of recombination in other organisms suggested the likelihood that nonhomologies could be included in regions of heteroduplex DNA. In yeast, substantial deletion mutations have been observed to participate in meiotic gene conversion (FINK and STYLES 1974; LAWRENCE *et al.* 1975). Transformation of pneumococcus with DNA from deletion mutants results in introduction of the deletions with high efficiency (LATASTE, CLAVERYS and SICARD 1980; CLAVERYS, ROGER and SICARD 1980). Pneumococcal transformation has been shown to proceed via a mechanism that involves single strand insertion with the formation of heteroduplex DNA (FOX and ALLEN 1964). In bacteriophage T4, three-factor crosses in which the central marker is a deletion exhibit localized negative interference (DOERMANN and PARMA 1967; BERGER and WARREN 1969), and evidence has been reported for the inclusion of very small deletions in heteroduplex regions (DRAKE 1966). Finally, phage that are heterozygous for deletion mutations have been observed among the progeny that emerge from crosses of the nonpermuted rhizobiophage 16-3 (DALLMANN, OROSZ and SAIN 1979; OROSZ, ROSTÁS and HOTCHKISS 1980; OROSZ, PÁY and DALLMANN 1980). This latter observation is, to date, the most direct evidence for the proposal that, on at least some occasions, recombination proceeds by a mechanism that can include nonhomologies in regions of heteroduplex DNA.

Single strand DNA might serve as a suitable substrate for formation of heteroduplex DNA which contains nonhomologies: The formation of heteroduplex DNA by branch migration of the symmetric two-strand crossover structure suggested by HOLLIDAY (1964) is at least in principle a passive one, and it is this feature that in theory renders branch migration sensitive to nonhomologies. Formation of heteroduplex DNA that includes a nonhomology should require a substantial amount of energy, since it would entail leaving a considerable number of bases unpaired. Part of this energy requirement could be met by a mechanism that gives rise to single strand DNA capable of strand transfer. One possible way in which such DNA might be generated in the course of a λ infection, and by which that single strand DNA could form a region of heteroduplex DNA which contains a nonhomology, is illustrated in a cartoon shown in Figure 4. In this cartoon, which incorporates features previously suggested by HOTCHKISS (1974a) and by MESELSON and RADDING (1975), single strand DNA is generated when a replication bubble encounters a nick in the uncopied strand of a DNA molecule. This single strand DNA would contain, by virtue of the mechanism of its formation, a free 3′ end. Single strand DNA with a free 3′ end could also be generated if a normal replication fork were to encounter a nick in duplex DNA. The action of the exonuclease encoded by one of the *red* genes of λ (CARTER and RADDING 1971) could play a role in generating

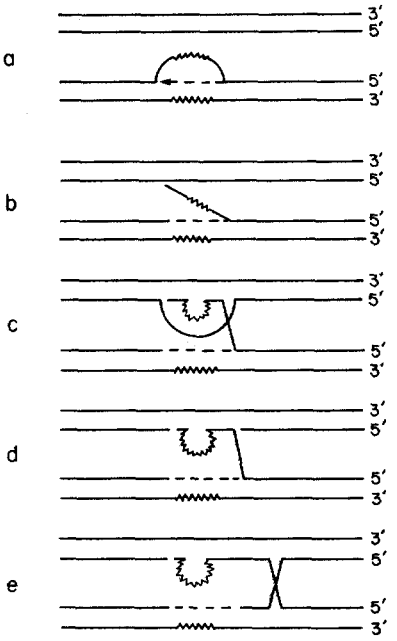


FIGURE 4.—A cartoon of how heteroduplex DNA containing a nonhomology might be formed. (a) A segment of single-stranded DNA is displaced by DNA synthesis. (b) When a nick in the displaced strand is encountered, single-stranded DNA with a free 3′ end is formed. (c) This single-stranded DNA can invade a second duplex molecule, displacing a single strand loop. (d) Degradation of the single strand loop allows formation of a stable one-strand crossover structure, which contains a nonhomology (in this case, a deletion) in a stretch of heteroduplex DNA. (e) This one-strand crossover structure can be converted to a two-strand, HOLLIDAY-type crossover structure.

or extending single strand sequences with 3′ ends. The free 3′ end could serve as a substrate for *recA*-promoted invasion of duplex DNA, as has been observed in various studies of the *in vitro* activities of the *recA* protein (KAHN *et al.* 1981; COX and LEHMAN 1981; WEST, CASSUTO and HOWARD-FLANDERS 1981). Alternatively, it could serve as the substrate for some still unidentified strand transfer activity, such as must be encoded by the *red* genes of λ. Finally, the one strand asymmetric crossover structure initially formed could potentially be converted to the symmetric two-strand crossover structure suggested by HOLLIDAY (MESELSON and RADDING 1975).

The model presented is highly speculative and is described here for purposes of illustration. The results of SODERGREN and FOX (1979) provided support for the proposal that branch migration cannot penetrate a region of nonhomology. These observations were based on an examination of the progeny that emerged from crosses performed under conditions restricting DNA synthesis. It remains possible that, in these experiments, heteroduplex regions that included nonhomologies were formed, but that they were present in products that, in the absence of subsequent replication, failed to satisfy the requirements for packaging into viable phage. It is also possible, as we have suggested, that DNA replication may be required for formation of such structures. The results presented in this

work indicate, albeit in a circumstantial way, that such heteroduplex DNA may be formed in λ crosses when DNA replication is unimpeded. Conclusive evidence that such structures are actually formed will require a direct examination of the immediate products of recombination.

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LITERATURE CITED

AMATI, P. and M. MESELSON, 1965 Localized negative interference in bacteriophage λ. Genetics **51:** 369–379.

BENZ, W. C. and H. BERGER, 1973 Selective allele loss in mixed infections with T4 bacteriophage. Genetics **73:** 1–11.

BERGER, H. and A. J. WARREN, 1969 Effects of deletion mutations on high negative interference in T4D bacteriophage. Genetics **63:** 1–5.

BÜCHEL, D. E., B. GRONENBORN and B. MÜLLER-HILL, 1980 Sequence of the lactose permease gene. Nature **283:** 541–545.

CARTER, D. M. and C. M. RADDING, 1971 The role of exonuclease and β protein of phage λ in genetic recombination. II. Substrate specificity and the mode of action of λ exonuclease. J. Biol. Chem. **246:** 2502–2512.

CLARK, A. J., 1974 Progress toward a metabolic interpretation of genetic recombination of *Escherichia coli* and bacteriophage λ. Genetics **78:** 259–271.

CLAVERYS, J. P., M. ROGER and A. M. SICARD, 1980 Excision and repair of mismatched base pairs in transformation of *Streptococcus pneumoniae*. Mol. Gen. Genet. **178:** 191–201.

COX, M. M. and I. R. LEHMAN, 1981 Directionality and polarity in recA protein-mediated branch migration. Proc. Natl. Acad. Sci. USA **78:** 6018–6022.

DALLMANN, G., L. OROSZ and B. SAIN, 1979 Restriction mapping of DNA of temperate *Rhizobium meliloti* phage *16-3*: comparison of genetic and physical maps indicates a long, genetically silent chromosomal arm. Mol. Gen. Genet. **176:** 439–448.

DAVIS, R. W., D. BOTSTEIN and J. R. ROTH, 1980 *Advanced Bacterial Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

DOERMANN, A. H. and D. H. PARMA, 1967 Recombination in bacteriophage T4. J. Cell. Physiol. **70** (Suppl. 1): 147–164.

DRAKE, J. W., 1966 Heteroduplex heterozygotes in bacteriophage T4 involving mutations of various dimensions. Proc. Natl. Acad. Sci. USA **55:** 506–512.

FINK, J. R. and C. A. STYLES, 1974 Gene conversion of deletions in the *HIS4* region of yeast. Genetics **77:** 231–244.

FOWLER, A. V. and I. ZABIN, 1977 The amino acid sequence of β-galactosidase of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **74:** 1507–1510.

FOX, M. S., 1978 Some features of genetic recombination in procaryotes. Annu. Rev. Genet. **12:** 47–68.

FOX, M. S. and M. K. ALLEN, 1964 On the mechanism of deoxyribonucleate integration in *Pneumococcal* transformation. Proc. Natl. Acad. Sci. USA **52:** 412–419.

FOX, M. S., C. S. DUDNEY and E. J. SODERGREN, 1979 Heteroduplex regions in unduplicated bacteriophage λ recombinants. Cold Spring Harbor Symp. Quant. Biol. **43:** 999–1007.

HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. **5:** 282–304.

HOTCHKISS, R. D., 1974a Molecular basis for genetic recombination. Genetics **78:** 247–257.

HOTCHKISS, R. D., 1974b Models of genetic recombination. Annu. Rev. Microbiol. **28:** 445–468.

KAHN, R., R. P. CUNNINGHAM, C. DASGUPTA and C. M. RADDING, 1981 Polarity of heteroduplex formation promoted by *Escherichia coli* recA protein. Proc. Natl. Acad. Sci. USA **78:** 4786–4790.

KAISER, A. D., 1955 A genetic study of the temperate coliphage λ. Virology **1:** 424–443.

LANGLEY, K. E., M. R. VILLAREJO, A. V. FOWLER, P. J. ZAMENHOF and I. ZABIN, 1975 Molecular basis of β-galactosidase complementation. Proc. Natl. Acad. Sci. USA **72:** 1254–1257.

LATASTE, H., J. P. CALVERYS and A. M. SICARD, 1980 Physical and genetic characterization of deletions in *Streptococcus pneumoniae*. J. Bacteriol. **144:** 422–424.

LAWRENCE, C. W., F. SHERMAN, M. JACKSON and C. A. GILMORE, 1975 Mapping and gene conversion studies with the structural gene for iso-1-cytochrome C in yeast. Genetics **81:** 615–629.

MAKIN, V., W. SZYBALSKI and F. R. BLATTNER, 1982 Asymmetric effects of deletions and substitutions on high negative interference in coliphage lambda. Genetics **102:** 299–317.

MALAMY, M. H., M. FIANDT and W. SZYBALSKI, 1972 Electron microscopy of polar insertions in the *lac* operon of *Escherichia coli*. Mol. Gen. Genet. **119:** 207–222.

MCMILIN, K. D. and V. E. A. RUSSO, 1972 Maturation and recombination of bacteriophage lambda DNA molecules in the absence of DNA duplication. J. Mol. Biol. **68:** 49–55.

MESELSON, M., 1967a The molecular basis of genetic recombination. pp. 81–104. In: *Heritage from Mendel*, Edited by A. BRINK. University of Wisconsin Press, Madison, WI.

MESELSON, M., 1967b On the mechanism of genetic recombination between DNA molecules. J. Mol. Biol. **9:** 734–745.

MESELSON, M., 1972 Formation of hybrid DNA by rotary diffusion during genetic recombination. J. Mol. Biol. **71:** 795–798.

MESELSON, M. and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72:** 358–361.

MESELSON, M. and J. WEIGLE, 1961 Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Natl. Acad. Sci. USA **47:** 857–868.

MORRISON, S. L. and D. ZIPSER, 1970 Polypeptide products of nonsense mutations. I. Termination fragments from nonsense mutations in the Z gene of the *lac* operon of *Escherichia coli*. J. Mol. Biol. **50:** 359–371.

OROSZ, L., A. PÁY and G. DALLMANN, 1980 Heterozygosis of phage 16-3 of *Rhizobium meliloti*: moderate level of mismatch repair or gene conversion. Mol. Gen. Genet. **179:** 163–167.

OROSZ, L., K. ROSTÁS and R. D. HOTCHKISS, 1980 A comparison of two-point, three-point and deletion mapping in the C cistron of rhizobiophage 16-3, with an explanation for the recombination pattern. Genetics **94:** 249–263.

SIGAL, N. and B. ALBERTS, 1972 Genetic recombination: the nature of a crossed strand-exchange between two homologous DNA molecules. J. Mol. Biol. **71:** 789–793.

SIGNER, E. R. and J. WEIL, 1968 Recombination in bacteriophage λ. I. Mutants deficient in general recombination. J. Mol. Biol. **34:** 261–271.

SODERGREN, E. J. and M. S. FOX, 1979 Effects of DNA sequence non-homology on formation of bacteriophage lambda recombinants. J. Mol. Biol. **130:** 357–377.

STAHL, F. W., S. CHUNG, J. CRASEMANN, D. FAULDS, J. HAEMER, S. LAM, R. E. MALONE, K. D. MCMILIN, Y. NOZU, J. SIEGEL, J. STRATHERN and M. STAHL, 1973 Recombination, replication and maturation in phage lambda. pp. 487–503. In: *Virus Research*, Edited by C. F. FOX and W. S. ROBINSON. Academic Press, New York.

STAHL, F. W. and M. M. STAHL, 1974 *Red*-mediated recombination in bacteriophage lambda. pp. 407–419. In: *Mechanisms in Recombination*, Edited by R. F. GRELL. Plenum Press, New York.

STAHL, F. W. and M. M. STAHL, 1977 Recombination pathway specificity of chi. Genetics **86:** 715–725.

THOMPSON, B. J., M. N. CAMIEN and R. C. WARNER, 1976 Kinetics of branch migration in double-stranded DNA. Proc. Natl. Acad. Sci. USA **73:** 2299–2303.

TRIMAN, K. L., D. K. CHATTORAJ and G. R. SMITH, 1982 Identity of a Chi site of *Escherichia coli* and Chi recombinational hotspots of bacteriophage λ. J. Mol. Biol. **154:** 393–398.

VISCONTI, N. and M. DELBRÜCK, 1953 The mechanism of genetic recombination in phage. Genetics **38:** 5–33.

WARNER, R. C., R. A. FISHEL and F. C. WHEELER, 1978 Branch migration in recombination. Cold Spring Harbor Symp. Quant. Biol. **43:** 957–968.

WEST, S. C., E. CASSUTO and P. HOWARD-FLANDERS, 1981 Heteroduplex formation by recA protein: polarity of strand exchanges. Proc. Natl. Acad. Sci. USA **78:** 6149–6153.

WHITE, R. L. and M. S. FOX, 1974 On the molecular basis of high negative interference. Proc. Natl. Acad. Sci. USA **71:** 1544–1548.

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