SOME PROPERTIES OF SITE-SPECIFIC AND GENERAL RECOMBINATION INFERRED FROM INT-INITIATED EXCHANGES BY BACTERIOPHAGE LAMBDA

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ABSTRACT

The site-specific recombination at the attachment site for prophage integration might proceed by two general mechanisms: (1) a concerted reaction without a free intermediate; (2) a sequential mechanism differing from typical general recombination only by an inability of the cross-strand intermediate structure to migrate into the region of nonhomology adjacent to the attachment site. The blocked-migration, sequential model predicts frequent genetic exchange in the *int xis* region near the attachment site if Int-mediated recombination occurs between λ phage with homologous attachment sites. We find such additional *int xis* exchanges, but only at very low frequency (1% of the Int-mediated recombination). We conclude that the resolution point only rarely moves away from the initial crossover point specified by Int and, therefore, that the Int reaction is mainly concerted. We interpret the rare additional *int xis* recombinants as indicative of occasional branch migration from an initial Int-mediated crossover. The frequency of the rare *int xis* recombinants is not simply related to distance from the attachment site to an *int*⁻ or *xis*⁻ mutation, suggesting that the heteroduplex distance is often at least a gene in length. The frequency of these additional exchanges is also not a strong function of distance between two mutations; from this we conclude that the resolution to the observed recombinant structure in the sequential cases occurs often by mismatch repair. We have found no marked effect of mutations in the bacterial *recA*, *recB*, *recC*, *recF*, or *recL* genes on the frequency of the *int xis* recombinants; this may indicate that none of these genes specifies a product uniquely required for resolution of a cross-strand intermediate.

THERE are two general biochemical models for genetic recombination. In the first, breakage and joining occurs in a single step without an intermediate free of the enzyme system responsible for recombination—a concerted mechanism. In the second, recombination involves distinct stages of initiation, DNA branch migration and resolution—a sequential mechanism. General recombination proceeds by the sequential route (see HOLLIDAY 1974, RADDING 1978 for reviews). The site-specific recombination characteristic of prophage integration seems to be a likely candidate for a concerted mechanism because of the highly localized exchange (FIGURE 1) (see ECHOLS 1971, WEISBERG, GOTTESMAN and

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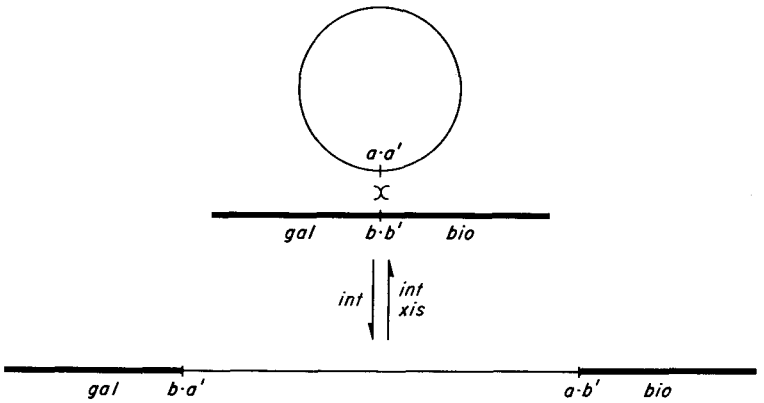

FIGURE 1.—The integration-excision event for phage λ. Site-specific recombination between the phage attachment site $a{\cdot}a'$ and the host attachment site $b{\cdot}b'$ (integrative recombination), directed by the phage Int protein, inserts the viral DNA. The inserted λ DNA is flanked by the recombinant prophage attachment sites $b{\cdot}a'$ and $a{\cdot}b'$. Site-specific recombination between these prophage attachment sites (excisive recombination), directed by the phage Int and Xis proteins, detaches the viral DNA. The central · in each attachment site represents a 15-base sequence that is a "common core" to each of the sites; each arm of the attachment sites—a,a',b,b'—has a different sequence (LANDY and Ross 1977).

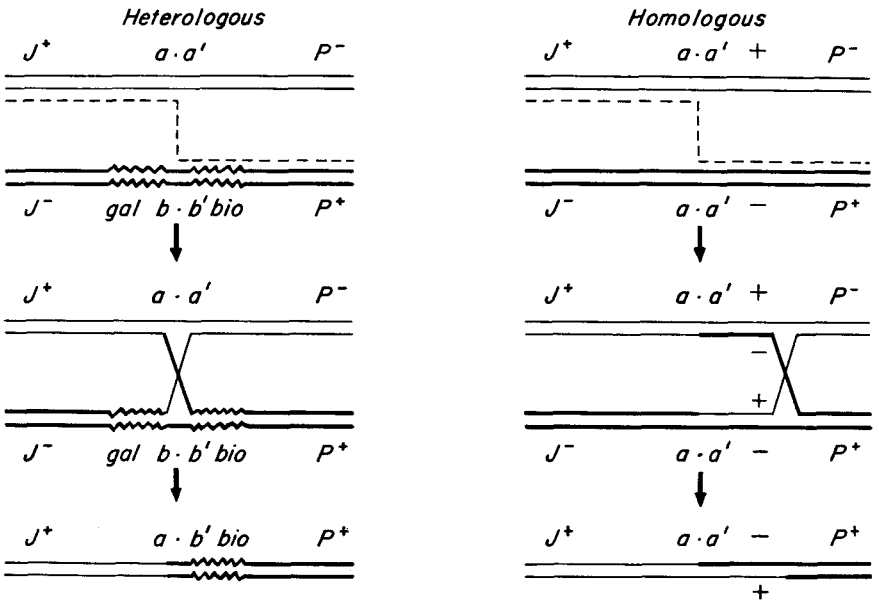

FIGURE 2.—Site-specific recombination between two phage. The exchange on the left is analogous to prophage integration; integrative recombination ($a{\cdot}a' \times b{\cdot}b'$) is recognized by the exchange of markers on opposite sides of the attachment site [*e.g.*, as $J{+}P{+}$ recombinants (ECHOLS 1970)]. For such an exchange, migration of a cross-strand intermediate might be blocked by the inhomologous DNA adjacent to the initiation point, resulting (after isomerization and cleavage) in the localized $J{+}a{\cdot}b'P{+}$ recombinant shown below. For the homologous exchange ($a{\cdot}a' \times a{\cdot}a'$) shown on the right, the postulated cross-strand intermediate should be free to migrate, generating a heteroduplex including nearby markers in the *int xis* region. If strand migration occurs, the $J{+}P{+}$ phage should include outside (+) recombinants not found in a completely localized crossover.

GOTTESMAN 1977 for reviews). However, the site localization might be achieved by sequence-specific initiation to a Holliday-type structure (defined by the phage Int protein), together with an inability of the exchanged strands to migrate away from the initiation point because of nonhomology between phage and host DNA to either side (Figure 2). If this were true, then site-specific and general recombination might be closely similar in mechanism, a possibility reinforced by evidence for specific initiation sites for general recombination (CATCHESIDE 1974, MALONE *et al.* 1978).

An expectaton of the "blocked-migration," sequential model for site-specific recombination is the appearance of genetic exchange in nearby regions if the attachment sites are homologous (Figure 2). For phage λ, this possibility can be readily tested by the use of *int*⁻ or *xis*⁻ mutations, which are located from 0.1 to 1.3 kilobases from the attachment site. In this paper, we report that such additional exchanges occur, but rarely, and argue for a predominantly concerted mechanism. ENQUIST, NASH and WEISBERG (1979) have drawn similar conclusions from an independent study.

The additional exchanges in the *int xis* region are presumably initiated at the attachment site, but are resolved elsewhere, far enough away to suggest resolution mechanisms identical to those found in general recombination. Some inferences are therefore possible about post-initiation events of general recombination, heteroduplex formation and resolution. Our beginning analysis along these lines is also presented here.

MATERIALS AND METHODS

Bacterial and bacteriophage: The *E. coli* and bacteriophage λ strains used in this investigation and their relevant genetic characteristics are listed in Tables 1 and 2.

TABLE 1

Bacterial strains

Strain	Relevant properties for this work	Reference
C600	Permissive host (*suII*+) for λ *am* mutations	CAMPBELL (1961)
R131	*recA13* derivative of RW842:nonpermissive (*su*⁻) host for λ *am* mutations; used to score *int xis* genotype of λ	ENQUIST and WEISBERG (1977)
R143	*recA13* derivative of W3101*su*⁻: standard strain for λ crosses	LEDERBERG (1960)
R144	*recA13* derivative of W3101*suII*+	
AB2463	*recA13* derivative of AB1157:standard strain for λ crosses in double *rec*⁻ hosts	HOWARD-FLANDERS and THERIOT (1966)
R166	*recA56* derivative of AB1157	
JC5495	*recA13 recB21* derivative of AB1157	WILLETTS and CLARK (1969)
JC5544	*recA13 recC22* derivative of AB1157	WILLETTS and CLARK (1969)
R169	*recA56 recF143* derivative of AB1157 (by way of JC8411)	HORII and CLARK (1973)
R165	*recA56 recL152* derivative of AB1157 (by way of JC8471)	HORII and CLARK (1973)
JC8111	*RecB21 recF143* derivative of AB1157	HORII and CLARK (1973)

The strains with "R" prefixes were made *recA*⁻ for this work—the reference is to the *rec*+ derivative; other strains are described in the reference listed.

TABLE 2

Phage strains

Genotype	Relevant properties for this work	Reference
Aam32, Jam6, Nam7, Pam80	Nonsense mutations used to score frequency of site-specific recombination	CAMPBELL (1961)
redB114, redXB3	Mutations blocking λ general recombination	SHULMAN *et al.* (1970)
int2	Missense mutation in the *int* gene	GINGERY and ECHOLS (1967)
int29	Nonsense mutation in the *int* gene	J. ZISSLER—see GUARNEROS and ECHOLS (1970)
int6	Missense mutation in the *int* gene	GOTTESMAN and YARMOLINSKY (1968)
int2271, int405	Nonsense mutations in the *int* gene	ENQUIST and WEISBERG (1977)
xis1	Missense mutation in the *xis* gene	GUARNEROS and ECHOLS (1970)

Media: The medium for bacterial growth and phage crosses was Tryptone broth (10 g of Difco Tryptone and 5 g of NaCl per l) supplemented with 0.2% maltose and 0.02% yeast extract. The plating medium was either Tryptone broth-agar (Tryptone broth with 1.2% agar or 0.7% soft agar overlayer) or tetrazolium (TTC)-agar plates with 1% galactose to score the *int* or *xis* genotype (ENQUIST and WEISBERG 1977).

Phage crosses: Bacteria were grown in supplemented Tryptone broth to a density of about 5×10^8 cells per ml. Phage were added at a multiplicity of five phage per cell for each parent. After a 20 min adsorption at room temperature, the infected cells were diluted 100-fold into supplemented Tryptone broth and incubated at 37° for 90 min; chloroform was added and the culture assayed for parental and recombinant phage. Parental phage carrying *am* mutations were scored as plaque-formers on C600*su*+; *am*+ recombinants derived from site-specific recombination (because all general recombination was blocked by mutation) were measured as plaque formers on R131 *su*⁻*recA*⁻; and *int*+*xis*+*am*+ recombinants were detected as red plaque-formers on R131 *su*⁻*recA*⁻, using TTC galactose indicator plates as described by ENQUIST and WEISBERG (1977) (*int*+*xis*+ phage make red plaques on R131 indicator bacteria because the Int/Xis proteins of λ excise phage DNA inserted into an otherwise normal bacterial *gal* operon, reverting the *gal*⁻ insertion mutation). Data reported are the average of two or more separate experiments. We found variation up to ± 50% of the numbers reported, especially in crosses in which different hosts were compared, so that small differences are not significant.

RESULTS

Int-*dependent recombination outside the attachment site:* To test for branch migration in an Int-initiated exchange, we have measured the frequency of recombination in the *int xis* region adjacent to the attachment site. We have carried out two types of experiments diagrammed in Figures 2 and 3. Figure 2 shows a single marker experiment in which an Int-initiated exchange at the attachment site leads to *int*+*J*+*P*+ recombinants, in addition to the localized recombinant class of *int*⁻*J*+*P*+. Figure 3 shows a two-marker experiment involving an outside exchange between two *int*⁻ or *xis*⁻ mutations, dependent on an initiating exchange at the attachment site; the interpretation of this experiment

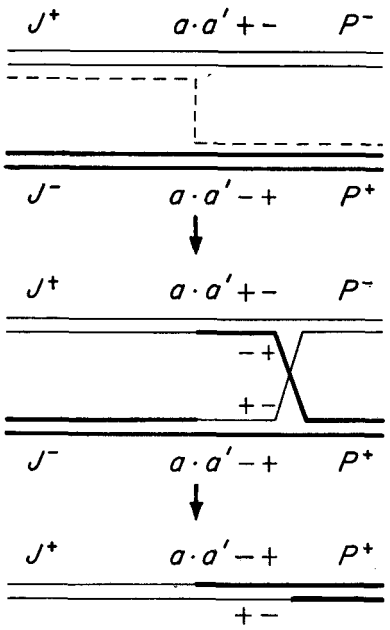

FIGURE 3.—Homologous exchange involving two nearby markers. If both markers are in a heteroduplex region (as shown), the production of $J+(+\!+)P+$ recombinants presumably requires mismatch repair of the heteroduplex; $J+(+\!+)P+$ recombinants might also result from a cleavage of the cross-strand intermediate (after isomerizaton) between the two markers.

is more complicated because a $J^+int^+xis^+P^+$ genotype requires either cleavage of the cross-stranded intermediate between the two int^- or xis^- mutations, or mismatch repair of the heteroduplex containing both mutations. In carrying out the two-marker experiment, we have used xis^- as one of the markers so that we can supply Int protein from the int^+xis^- phage.

The interference experiment of Figure 3 is most useful to show the dependence of outside recombination on Int protein—the basic assumption of the rest of the

TABLE 3

Evidence for Int-initiated outside exchanges

Cross	Int-mediated exchanges [am^+/total ($\times 10^{-2}$)]	Outside exchanges [$int^+xis^+am^+$/total ($\times 10^{-4}$)]
Aam32 xis1/Pam80 int am29	1.1	0.6
Jam6 int2/Pam80 int am29	0.01	<0.01
Jam6 int2/Pam80 int am29 (sulI)	0.5	0.08

One parental phage carried the *Aam32* or *Jam6* nonsense mutation, located to the left of the attachment site; the other parent carried the *Pam80* mutation, located to the right of the attachment site (Figure 2 and Figure 3). General recombination was blocked because both parental phage carried the *redB*114 mutation, and the host was *R143su⁻recA⁻* (or *R144sulI⁺recA⁻* for line 3); am^+ recombinants should therefore reflect the frequency of Int-mediated recombination events (ECHOLS, GINGERY and MOORE 1968; WEIL and SIGNER 1968). The Xis protein is not required for site-specific recombination between homologous attachment sites (ECHOLS 1970).

analysis (Table 3). Rare recombination outside the attachment site (between *int29* and *xis1*) can be detected (frequency about 10^{-4}; line 1). Exchanges in this region do not occur if both parents are *int*$^-$, so that Int protein is not supplied (line 2) [the *int29/int2* distance is comparable to the *int29/xis1* distance (Figure 4)]. Outside exchanges are found for the *int29/int2* cross if the *int29* nonsense mutation is suppressed in a *sulI*+ host, providing a reduced level of active Int protein. Thus we conclude that Int-mediated recombination is required for the outside exchanges. Additional control experiments to show dependence on active Int protein and the host components for integrative recombination have also been carried out, but are not reported here because similar experiments are presented in ENQUIST, NASH and WEISBERG (1979).

Distance effects on recombination outside the attachment site—a measure of heteroduplex length: If the recombination in the *int xis* region is derived from an Int-initiated crossover, an estimate of the frequency and distance of branch migration from the attachment site can be obtained from an experiment of the type shown in Figure 2, in which the distance of an *int*$^-$ or *xis*$^-$ mutation from the attachment site is varied. The results of such an experiment are shown in Figure 4. The absence of a strong distance effect on the frequency of *int*+*xis*+

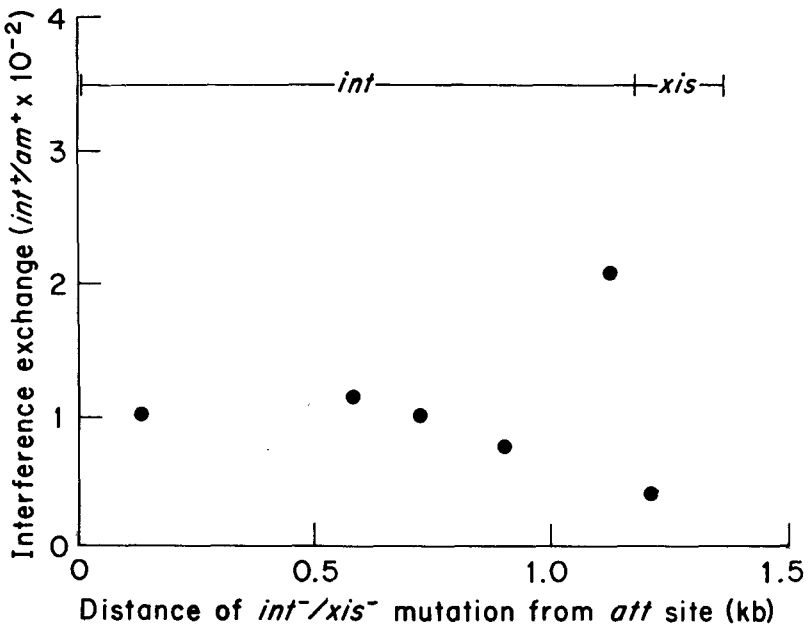

FIGURE 4.—Distance effects on exchanges outside the attachment site. The frequency of *int*+*xis*+ recombinants among total site-specific (*am*+) recombinants is plotted as a function of estimated distance of the mutation from the attachment site. All crosses are of the type shown in Figure 2; the *int*$^-$ or *xis*$^-$ parent carried the *Aam32 or Jam6* mutation, and the *int*+ parent carried the *Nam7* mutation. The host was R143 *recA* and each phage carried the *redB114* or *redXB3* mutation, so that all general recombination was blocked. The locations of the *int*$^-$ or *xis*$^-$ mutations were taken from the mapping data of ENQUIST and WEISBERG (1977). The mutations used were (from left to right on the figure): *int2271*, *int405*, *int6*, *int29*, *int2*, and *xis1*.

recombinants indicates that the heteroduplex often extends through the *int xis* region (a typical gene in length). The slight overall gradient may indicate that the most probable length is somewhat less than a gene, if the high point at the right end of the *int* gene is ascribed to a resolution preference (presumably in mismatch repair).

If heteroduplexes extend at least several hundred base pairs (as indicated by the data in Figure 4), the low frequency of int^+xis^+ for even the closest marker (about 100 base pairs) suggests that branch migration away from the attachment site is a rare event. We conclude that site-specific recombination is mainly a concerted reaction, although rare sequential events do occur, involving the extensive heteroduplex regions typical of general recombination. (Alternative interpretations are considered in the DISCUSSION.) In their study, ENQUIST, NASH and WEISBERG (1979) found a steeper gradient with distance than we (for the three markers they used), but also conclude that their results are incompatible with a freely migrating cross-strand intermediate.

Interference exchanges between two outside markers—an indication of mismatch repair: As noted above, a cross of the type shown in Figure 3 can yield int^+xis^+ recombinants by two mechanisms: cleavage of the cross-strand intermediate between the two markers or mismatch repair of a longer heteroduplex containing both markers. We have interpreted the distance results of the previous section in terms of frequent formation of a heteroduplex extending throughout the *int xis* region. If this is the case, mismatch repair is likely to be involved frequently in the resolution of two-marker heteroduplexes to int^+xis^+. For a mismatch repair mechanism, we expect little (if any) effect of distance between the int^- or xis^- mutations on the frequency of int^+xis^+. The data of Table 4 show that this expectation is fulfilled; the frequency of int^+xis^+ is about the same for markers 1.1 kilobases apart (*int2271/xis1*) as for markers estimated as within 0.1 kilobases (*int2/xis1*) (see Figure 4). In addition, the frequency of int^+xis^+ in the two marker cross is comparable to that found in the one marker cross (compare Table 4 and Figure 4). From these results we conclude that mismatch repair occurs frequently in heteroduplex structures.

TABLE 4

Lack of distance effect in two-marker outside exchanges

Cross	Int-mediated exchanges [am^+/total ($\times 10^{-2}$)]	Outside exchanges [$int^+xis^+am^+/am^+$ ($\times 10^{-2}$)]
int2271/xis1	0.9	1.0
int405/xis1	0.9	1.1
int6/xis1	0.5	1.4
int29/xis1	0.6	1.0
int2/xis1	1.4	1.1

The int^- parent carried the *Aam32* or *Jam6* mutation to the left of the attachment site; the *xis1* parent carried the *Pam80* mutation to the right of the attachment site. As for Table 3 and Figure 4, general recombination was blocked by the use of red^- phage and the R143*recA*$^-$ host.

TABLE 5

Outside exchanges in rec⁻ strains

Host genotype	Int-mediated exchanges [am^+/total ($\times 10^{-2}$)]	Outside exchanges [$int^+xis^+am^+/am^+$ ($\times 10^{-2}$)]
recA13	1.2	0.8
recA56	1.4	0.8
recA56 recC22	2.1	0.6
recA13 recB21	1.3	0.5
recA56 recF143	2.0	0.9
recA56 recL152	2.2	0.6
recB21 recF143	2.2	1.1

Phage parents were *Jam6 int2* and *Pam80 xis1*; both parental phage carried the *redB114* mutation to block phage general recombination.

Search for host mutations affecting interference exchanges: Based on the arguments given above, the resolution of a two-marker cross (such as *int2* × *xis1*) to an int^+xis^+ recombinant phage should be dependent on cleavage of the cross-strand intermediate and mismatch repair to int^+xis^+. If a single bacterial gene specifies an obligatory enzyme for either of these processes, an analysis of bacterial mutants might reveal the gene involved. Therefore we have initiated a study of host mutations for possible effects on the additional *int xis* exchange. Because of the rarity of the int^+xis^+ recombinants ($\sim 10^{-4}$), this effort is complicated by a need to eliminate bacterial general recombination. We have done this by making all of the host strains $recA^-$ and analyzing the effect of an additional mutation.

The results of crosses involving rec^- mutations are given in Table 5. There is no drastic effect on the frequency of exchanges in the *int xis* region; small differences are difficult to interpret because of likely differences in the physiological state of the different double mutants as a result of impaired growth. There is also no marked effect of $recA^+$ in the $recB^-$ $recF^-$ double mutant (line 7); thus RecA protein by itself is probably not a major contributor to either formation or resolution of the Int-derived heteroduplex structure. We infer that none of the known *rec* genes specifies a protein uniquely required for resolution of a cross-strand intermediate.

DISCUSSION

Mechanism for integrative recombination: We have shown that genetic exchange between λ phage occurs in the *int xis* region, dependent on the capacity of phage DNA to undergo integrative recombination at the attachment site. We consider the most likely explanation for these additional exchanges to be branch migration from the attachment site of an Int-initiated cross-strand intermediate. If the branch migration interpretation is correct, we can infer that the heteroduplexes are often at least a gene in length, a conclusion consistent with previous estimates for general recombination (WHITE and FOX 1974; WILDENBERG and MESELSON 1975). Since heteroduplexes are frequently long, if formed at all, the

rarity of outside exchange for even the closest marker implies that strand "escape" from the attachment site is infrequent. Thus we conclude that site-specific recombination normally occurs by a "concerted mechanism" in which there is not free migration of a cross-strand intermediate. Integrative recombination might occur most often by a double-strand exchange in which a cross-strand intermediate is not normally formed, or the Holliday-type structure might be formed, but resolved without the release of a free intermediate. In either case, site-specific recombination involves a mechanism distinct from general recombination in more ways than localized initiation.

There are alternative interpretations to those presented above, which we consider less likely but cannot exclude. One can argue that the Int reaction is normally sequential, but that efficient branch migration requires a completely active general recombination system. Because our experiments (and those of ENQUIST, NASH and WEISBERG 1979) were of necessity done under conditions in which general recombination is blocked, our view of the Int reaction might be biased by the experimental system. We have dismembered the Rec system one component at a time (including RecA) (Table 5) and seen no effect. Thus we think that this viewpoint is probably not correct because it implies that all components of general recombination are required for branch migration.

From the opposite point of view, one can argue that the rare additional recombinants in the *int xis* region do not involve branch migration from an Int-initiated exchange, but are instead derived from a secondary cross-strand exchange near the attachment site. This additional exchange might occur because of a secondary Int site near the attachment site or because the Int reaction provides for proximity of the two DNA molecules (a synaptic event). In these models, the normal Int reaction becomes completely concerted (rather than 99%). We think that a secondary Int site near to, but distinct from, the attachment site is unlikely because the frequency of outside exchanges is not a simple function of distance from any point for either one- or two-marker crosses (Figure 4, Table 4, and additional data not shown). The synaptic model cannot be excluded; if correct, the major interpretative complexity is that initiation of general recombination is not completely bypassed in Int-mediated outside exchanges.

Mechanisms in general recombination: The importance of mismatch repair in phage recombination has been inferred from the formation of wild-type recombinants after infection by heteroduplex DNA molecules formed *in vitro* (a situation in which only mismatch repair is available) (SPATZ and TRAUTNER 1970; WILDENBERG and MESELSON 1975; WAGNER and MESELSON 1976). Although less direct, our experiments are done under conditions in which alternative modes of resolution can occur; our results suggest that mismatch repair is the major pathway for genetic exchange between very close markers.

We believe that we have developed an "Int-initiated, general-resolved" experimental system that will be useful for analyzing general recombination because effects on the initiation and resolution stages of general recombination can be separated. With this in mind, we have initiated an analysis of the role of host genes in the occurrence of Int-mediated outside exchanges.

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