Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4530–4534, August 1982
Biochemistry

Cointegrate formation by Tn*5*, but not transposition, is dependent on *recA*

(transposable elements/recombination/mechanism of transposition)

BERNARD J. HIRSCHEL*, DAVID J. GALAS†‡, AND MICHAEL CHANDLER†

*Department of Infectious Diseases, Cantonal Hospital, Geneva, Switzerland; and †Department of Molecular Biology, University of Geneva, Geneva, Switzerland

Communicated by Martin D. Kamen, April 2, 1982

ABSTRACT **We have studied the effect of the *recA*-dependent homologous recombination system of *Escherichia coli* on both Tn*5*-mediated cointegrate formation and Tn*5* transposition. We demonstrate here that, whereas transposition of Tn*5* is independent of the *recA* gene product (as has been shown by other workers), Tn*5*-mediated cointegrate formation is strongly dependent on *recA*. The structures of both the simple transposition products and the cointegrates formed in a *recA*$^-$ background seem to be the same as those produced in a *recA*$^+$ background. These results provide strong evidence that Tn*5* does not transpose via an obligate cointegrate intermediate and suggest that the *recA* effect on cointegrate formation is exerted during the process of transposition.**

Transposable genetic elements are capable of insertion into many distinct nonhomologous sites in the genomes of bacteria and phage. In addition, they are known to promote the fusion of replicons (cointegrate formation) and the formation of deletions and inversions of DNA (for recent reviews see refs. 1 and 2). It has been thought that most, if not all, of the recombination events induced by transposable elements are independent of homology-requiring recombination systems, first because the recombination events are between nonhomologous sequences, and second because transposition itself and several other transposition-related processes appear to occur with equal facility in wild-type cells and cells deficient in the *recA* gene product (3, 4). [An exception to this *recA* independence has recently been reported for the transposition of Tn*903* from phage λ to the *Escherichia coli* chromosome (5).]

Galas and Chandler (6) have proposed that transposable elements in bacteria can be largely classified into two groups. Certain elements, including Tn*3* and γδ (Tn*1000*), generate 5-base-pair repeats in the target DNA on insertion and appear to form unstable cointegrates as obligate intermediates in transposition. These cointegrates consist of donor and recipient replicons delimited by two directly repeated copies of the transposable element. This class of element encodes a site-specific recombination system that promotes recombination between the two directly repeated copies of the element (resolution of the cointegrate), and generates, on recombination, a copy of the donor and a copy of the recipient replicon, now also carrying the transposon (7–10).

The second class of element, which includes Tn*5*, Tn*9*, Tn*10*, and Tn*903*, can be considered compound elements because they are composed of a region of DNA encoding an antibiotic resistance determinant flanked by two elements that are themselves independently transposable. Elements in this class generate 9-base-pair repeats in the target DNA on insertion. They are also capable of fusing replicons. In contrast to cointegrate structures generated by the first class, those generated by Tn*9* (11) and Tn*5* (12, 13) are quite stable. The stability of the cointegrates, together with the observation that cointegrate structures are formed at a frequency lower than the transposition frequency in a *recA*$^-$ host (ref. 11; this work) has led us to propose that cointegrates are not obligate intermediates in the transposition of this second class of element. It seems likely that two pathways exist: one that leads to direct transposition and a second that results in replicon fusion.

Harshey and Bukhari (14) and Galas and Chandler (6) have proposed models of transposition that involve two pathways. We have suggested that the difference in the pathways for the two classes of elements lies in the final steps of the process: the Tn*3*-like elements form cointegrates whereas the compound elements have a strong preference for direct transposition but can also form cointegrates. The data that we present here demonstrating that cointegrate formation by Tn*5*, but not transposition, is strongly dependent on the *recA* gene of the host provide support for models of transposition that postulate independent or branching pathways for direct transposition and cointegrate formation. The data suggest that Tn*5* transposes almost entirely by a direct process. We argue further that it is likely that the *recA*-dependent step occurs during the process of transposition rather than after the end products are separated.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The *E. coli* strains and plasmids used in this work are listed in Table 1.

Chemicals and Media. All restriction enzymes were obtained from New England BioLabs or Boehringer Mannheim. Phage T4 DNA ligase was from New England BioLabs. Rich medium (15) was used throughout and supplemented, where appropriate, with various antibiotics at the following concentrations: kanamycin (Km), 20 μg/ml; streptomycin (Sm), 20 μg/ml; tetracycline (Tc), 25 μg/ml; nalidixic acid (Nal), 35 μg/ml.

Plasmid DNA Preparation. Plasmid DNA was prepared for analysis by the method of Clewell and Helinski (16). For screening of plasmids, the method of Birnboim and Doly (17) was used.

Construction of Tn*5*-KS. The Tn*5* used in this construction was isolated on pBR322 as described (18). We wished to insert the gene for Sm^R from R100 into the *Bam*HI site of Tn*5*. The Sm^R determinant can be isolated on a single *Bgl* II/*Bam*HI fragment (19). This fragment can be inserted directly into the single *Bam*HI site of Tn*5*. Because pBR322 also contains a single *Bam*HI site we partially digested pBR322::Tn*5* with *Bam*HI, heat-denatured the enzyme, and prepared the DNA for ligation. We ligated this DNA (about 2 μg) with an equivalent

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Km, kanamycin; Sm, streptomycin; Nal, nalidixic acid; Tc, tetracycline; R, resistant; kb, kilobases.

‡To whom reprint requests should be addressed at: Dept. of Molecular Biology, Univ. of Southern California, Los Angeles, CA 90007.

Table 1. Bacteria and plasmids

Strain	Relevant genotype and phenotype
BH16	LC245/pOX38/pBR322::Tn*5*-KS
BH17	GK30/pOX38/pBR322::Tn*5*-KS
BH28	DB1504/pBR322::Tn*5*-KS
BH29	LC445/pOX38::Tn*5*/pBR322
BH30	LC916/pOX38::Tn*5*/pBR322
BH31	LC102/pOX38::Tn*5*/pBR322
DB1504	Δ*trpE5* Δ(*lac–proB*)
DB1696	DB1504/pOX38::Tn*5*/pBR322
DB1706/3	DB1504/pBR322::Tn*5*
GK30 (derived from LC245)	*trpE985, gal1, recA, supE*
LC102	*leu, purE, trp, his, metA, ile, argG, proC, thi, ara, lac, xyl, mtl, gal, strA*, T6^R
LC245	*thyA, supE, gal-1*
LC445 (derived from LC102)	*leu, trp, metA, ile, argG, proC, thi, ara, lac, xyl, mtl, gal, strA, recA1*, T6^R
LC654	*thr, leu*/pLC80
LC799	*thr, leu, lac, thi*, Nal^R, P1^R, λ^R
LC839	LC445/pOX38
LC916	*thy, thi, met, pro, recA*, Rif^R

^{R}, Resistant; Rif, rifamycin; T6, P1, and λ are phages.

amount of the Sm^R *Bgl* II/*Bam*HI [3.5-kilobase (kb)] fragment [purified from pLC80 (19, 20)]. Plasmid DNA was prepared from several of the Km^R, Sm^R transformants and the insert was located, sized, and analyzed. The plasmid was then used to transform a strain containing pOX38, LC839 (*recA*$^-$), and was found to transpose Sm^R along with Km^R.

Transposition and Cointegrate Mating Assay. The donor strains were grown on plates containing Km and Tc. A single colony was picked to inoculate a culture of L broth (LB) supplemented with Km and Tc and grown overnight. The bacteria were pelleted, washed once with LB, diluted 1:100 into LB, and mixed with an equal volume of 1:100 dilution of a saturated culture of the recipient. The mating was allowed to proceed for 3–4 hr before the cells were pelleted and plated on selective plates.

Tests for retransfer of the cointegrates were performed by cross-streaking the various strains against LC916 or LC445 on selective plates. In the course of our experiments we found that the frequency of cointegrates in the population, when measured directly by selection among the exconjugants for Tc^R, Km^R, and Sm^R, was lower than that obtained by selection for Km^R and Sm^R followed by screening those resistant colonies for Tc^R. This discrepancy, presumably a plating efficiency effect, was consistently 3- to 5-fold in favor of the indirect method. This dictates that the indirect method be used to measure cointegrate formation. Exconjugants resistant to Km and Sm were scored for Tc sensitivity (transpositions) or Tc^R (cointegrates). Both classes were tested for their ability to retransfer these markers at high frequency in a subsequent mating. Only those clones that retransferred were scored as transpositions or cointegrates. Although this method of assessing transpositions and cointegrates may exclude those in which the insertion has occurred within the transfer genes of pOX38, it effectively excludes bacterial mutants.

In each mating 100 Nal^R exconjugants were tested for the presence of pOX38 by cross-streaking against the male-specific phage fd, and the frequency of transposition and cointegrate formation are expressed relative to the transfer of pOX38.

Fluctuation Test. A single colony of the donor strain was inoculated into liquid medium and grown overnight. This culture was then diluted into fresh medium and dispensed into a number (usually 54) of culture tubes (1.5 ml each) such that the estimated number of cells per tube was about 300. These cultures were then grown at 37°C for various times to obtain the number of cells per tube appropriate to the frequency to be measured. At this point 0.5 ml of a freshly saturated culture of the recipient, LC799, was added to 51 of the cultures. They were then incubated without active aeration for 30 min, then incubated with gentle aeration for 30 min. At the time the recipient cells were added, three of the tubes without the added recipient cells were diluted and plated to determine the viable count of the donors. The contents of the other tubes were then concentrated 10-fold and plated, 50 of them on rich medium containing Nal, Km, and Sm, and one on rich medium with Nal only. This last mating was used to assess the mating efficiency by testing 50 of the Nal^R exconjugants for sensitivity to phage fd.

The selective plates were incubated for 24 hr, scored, and then replicated onto Nal, Tc plates. The number of cultures from which no cointegrates (or transpositions) were found, $N(0)$, was used to calculate the average number of cointegrate formation (or transposition) events per culture, m, from the formula (21) $N(0)/N$ (total) $= e^{-m}$. The frequency of occurrence of these events is taken as frequency (per cell per generation) = $(m \ln 2)/$(no. of cells per culture).

Analysis of Transpositions and Cointegrates. The central segment of Tn5-KS carries a single *Bam*HI site (Fig. 1) as does pBR322 (25). It should be noted that the *Bam*HI/*Bgl* II junction within this element is no longer susceptible to *Bam*HI or *Bgl* II. The orientation of Tn5 within pBR322 has been previously determined (see above and ref. 18). Comparison of pBR322::Tn5 with pBR322::Tn5-KS shows that the smaller of the two *Bam*HI fragments is not affected by the insertion of the 3.5-kb Sm^R determinant. This fragment carries IS*50*-L. The larger of the two fragments must therefore carry IS*50*-R.

We have determined the structure of the cointegrates by comparison of restriction digests with those of pBR322::Tn5-KS as follows. The pBR322::Tn5-KS plasmid gives two fragments on digestion with *Bam*HI, each carrying one copy of IS*50* (Fig. 2). If either of the *Bam*HI fragments disappears on formation of the cointegrate, the IS*50* in that fragment has been used to mediate the cointegrate. If neither *Bam*HI fragment disappears, either the entire element is involved or multiple copies of the donor pBR322::Tn5-KS have been inserted. Digestion of the two cointegrates, 18.1 and 18.2, generated in a *recA* background showed that 18.2 lost the larger of the two pBR322::Tn5-KS *Bam*HI fragments (implicating IS*50*-R in cointegrate formation), whereas 18.1 retained both fragments. The smaller is

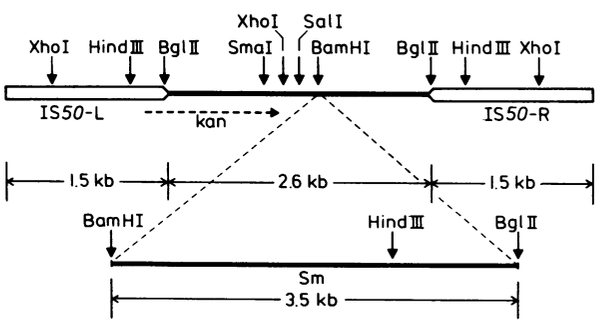

FIG. 1. Structure of the Tn*5* derivative Tn*5*-KS. This transposon, whose construction is described in the text, contains a segment (indicated as an insert in Tn*5*) of the plasmid R100.1 specifying resistance to Sm. The orientation of the flanking elements IS*50* is known from refs. 22 and 23. The structure and the positions of the restriction sites were taken from Rothstein and Reznikoff (24), Lane and Gardner (19), and our own unpublished observations. All sites shown have been verified in this work. *kan* indicates gene conferring Km^R.

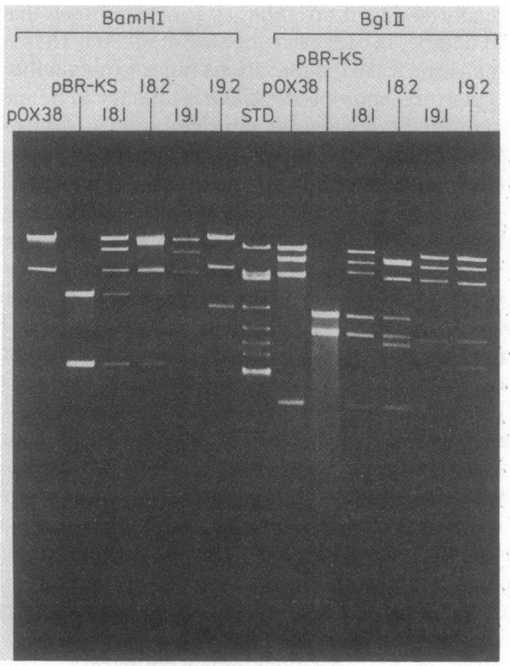

FIG. 2. Agarose gel (0.75%) electrophoresis of *Bam*HI- and *Bgl* II-digested plasmid DNA. The plasmid number designations are described in the text and in Fig. 3. pBR-KS is the plasmid pBR322::Tn*5*-KS described in the text. The size standard (STD.) is an *Eco*RI digest of a mixture of the plasmids R100.1 and pBR322 (intense band in the center of gel). The uppermost band in the STD. lane is 20.5 kb. The interpretation of these data is described in detail in *Materials and Methods*.

present in double amount. The donor pBR322::Tn5-KS has only two *Bgl* II sites (Fig. 2), within the IS*50* elements (Fig. 1). Digestion of the cointegrates with *Bgl* II (Fig. 2) showed that both 18.2 and 18.1 carry both fragments. While 18.2 carries these fragments in unimolar amounts, confirming the structure deduced from the *Bam*HI digest (Fig. 3), both fragments are present in greater than unimolar amount in 18.1. This implies that pBR322::Tn*5*-KS multimers are present in the cointegrate. Further analysis of these plasmids by using *Xho* I and *Sma* I (data not shown) resolved the possible ambiguities in interpretation. The structures determined by these results for cointegrates 12 and 18.2 are shown in Fig. 3. The number of fragments for each digest analyzed and their sizes are compatible only with the structures shown in Fig. 3. The transposition products (19.1 *recA*$^-$ and 19.2 *recA*$^+$) have been analyzed in a similar manner. Insertion of Tn5-KS results in an extra *Bam*HI fragment in pOX38 (Figs. 1 and 2), and two extra *Bgl* II fragments. Analyses of internal heteroduplexes ("snapback" structures) in the electron microscope have confirmed the structure of these molecules as deduced from restriction analysis (data not shown).

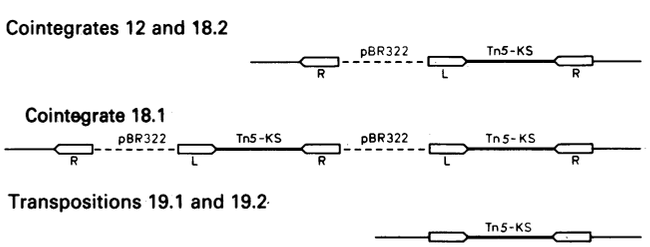

FIG. 3. Structures of the plasmids analyzed. The number designations are defined in the text. The broken line represents pBR322, the solid line pOX38, and the open boxes IS*50* elements. The latter are drawn to show their relative orientation. L and R represent the left and right copies of IS*50* as shown in Fig. 1.

RESULTS

We have used the plasmid pBR322, the sequence of which has been fully determined, and an F-derived conjugal plasmid, pOX38 (27), as donor or recipient replicons to detect Tn5-mediated cointegrate formation and transposition. The plasmids and the mating system have been described in refs. 11 and 13. The plasmid pBR322 is nontransmissible and nonmobilizable. It can, however, be transferred from cell to cell when fused with a conjugal plasmid (27). Neither pOX38 nor pBR322 carries any known transposable elements, and transfer of pBR322 by pOX38 is an extremely rare event (see below). Introduction of a transposable element into either of the replicons, however, results in an increase of several orders of magnitude in the frequency of transfer of pBR322 by pOX38 (11, 13, 26, 28). In the experiments reported here cointegrate formation was measured in two ways. In initial experiments (Table 2) we used a Tn5-insertion derivative of pOX38 to transfer pBR322. In this case, the transfer of pBR322 is a measure of the frequency of Tn5-mediated cointegrates in the population (unpublished). In the second type of experiment a Sm^R derivative of Tn5 carried by pBR322 was used as a donor and pOX38 as a recipient. This experiment can be used to measure both the frequency of Tn5 transposition (transfer of Km^R, Sm^R alone) and the frequency of Tn5-mediated cointegrate formation (transfer of Km^R, Sm^R, and Tc^R).

Cointegrate Formation and Transposition Between pBR322::Tn5 and pOX38. Although *recA* has been reported to have little effect on transposition of Tn5 (3), the effect on the formation of cointegrates is striking. In order to confirm that the effect of *recA* is really on the *formation* of cointegrates it would be useful to compare the effect of *recA* on both transposition and cointegrate formation in the same experiment. The ideal experiment for such a comparison is a mating experiment identical to those described above, but in which Tn5 is carried on pBR322 in the donor strain and pOX38 is the target replicon. Both transposition and cointegrate formation can be assayed by scoring exconjugants for Km^R, Tc^S(^{S}, sensitivity) and Km^R, Tc^R.

Experimental System. We found that the selection for Km^R among the exconjugants was not strong enough to measure transposition events at less than 10^{-7} in the population. When we plated more than about 5×10^7 (about 0.1 ml of saturated culture) recipients on a Km plate, many colonies grew that proved not to carry Tn5. This background interferes with the reliable estimation of the transposition frequency. To overcome

Table 2. Frequency of cointegrate formation between pOX38::Tn*5* and pBR322

Strain background	Titer, exconjugants per ml	Titer, cointegrates per ml	Frequency, cointegrates per cell per generation
LC445 (*recA*$^-$)	3.8×10^8	0.25	$4.5 (\pm 4.0) \times 10^{-10}$
LC916 (*recA*$^-$)	6.7×10^8	0.20	$2.0 (\pm 0.8) \times 10^{-10}$
LC102 (*recA*$^+$)	4.5×10^8	12	$1.8 (\pm 0.5) \times 10^{-8}$
DB1696 (*recA*$^+$)	2.2×10^8	15	$4.7 (\pm 1.7) \times 10^{-8}$
	Average ratio (*recA*$^+$/*recA*$^-$)		100

The measurements are the results of five parallel mating experiments for each strain. The frequencies are estimated from the *rate* of occurrence of these events—i.e., frequency in the population multiplied by ln 2 (ref. 29) and are given ± SD.

the problem we constructed and used a derivative of Tn5 that carries, in addition to the Km^R determinant, a Sm^R determinant. We inserted a segment of DNA that specifies Sm^R (a *Bam*HI/*Bgl* II fragment from the plasmid R100.1) into the unique *Bam*HI site of Tn5 (Fig. 1). The construction of this Km^R, Sm^R derivative of Tn5 (Tn5-KS) is described in *Materials and Methods*. In initial experiments using both Sm and Km for selection we found that more than 10^8 recipients could be plated without encountering significant background growth.

Frequencies of Transposition and Cointegrate Formation. In Table 3 we present the results of 10 independent experiments (using an almost isogeneic *recA*$^+$ and *recA*$^-$ pair of strains) in which the exconjugants from a mating in which the donor carried pOX38 and pBR322::Tn5-KS were selected for Km^R, Sm^R and then screened for Tc resistance (cointegrates) or sensitivity (transposition). The estimated frequencies of transposition, estimated from the titer of Km^R, Sm^R exconjugants, are essentially the same in the *recA*$^-$ and *recA*$^+$ background. The frequencies of cointegrate formation, however, differ by almost two orders of magnitude, a ratio identical to that found in the previous experiments.

Cointegrates between pOX38 and pBR322 are known to have an increased mating efficiency compared to pOX38 (11, 13, 30). We are not concerned with this effect here, however, because the corrections are identical for *recA*$^+$ and *recA*$^-$ strains.

In order to conclude definitively that cointegrate formation is largely dependent on the *recA* function, it is necessary to rule out the possibility that the difference in frequency we observe is caused by a difference in the viability of *recA*$^-$ and *recA*$^+$ cointegrate-carrying strains. We have investigated this possibility in two ways. First, we compared the growth rates of *recA*$^+$ and *recA*$^-$ strains carrying the same cointegrate with the same strains carrying only pOX38::Tn5-KS in mixed culture experiments. We detected no difference (data not shown). The second, and more sensitive, method we employed is the measurement of the frequencies by the fluctuation test of Luria and Delbrück (21). Because the frequencies are calculated from the fraction of cultures in which an event did *not* occur, we avoided any possible effect of differential growth rates or viability. The results are shown in Table 4. The frequencies obtained by this method are close to those reported above.

Structure of the Cointegrates. Although cointegrate formation is strongly inhibited by the absence of *recA* function, it does occur at low frequency in *recA*$^-$ strains. Several of these rare cointegrates produced in the *recA*$^-$ background were physically analyzed to determine whether they were true cointegrates mediated by Tn5 (the structures of 23 independent cointegrates produced in a *recA*$^+$ background are reported in ref. 13). We chose one example of a transposition (19.1) and one cointegrate (12) from a *recA*$^+$ donor (LC245), and one transposition (19.2) and two cointegrates (18.1, 18.2) from a *recA*$^-$ donor for analysis. Fig. 2 shows restriction enzyme digests of several of these. The relevant restriction sites in Tn5-KS are shown in Fig. 1. The analysis of the plasmid structures is outlined in *Materials and Methods* and in Fig. 2. The cointegrate structures determined are shown schematically in Fig. 3. The cointegrates were of two kinds: 12 (*recA*$^+$) and 18.2 (*recA*$^-$) were simple cointegrates mediated by IS*50*-R, and 18.1 (*recA*$^-$) was a more complex structure containing two copies of pBR322, two copies of Tn5-KS, and one extra copy of IS*50*-R. This latter structure was confirmed by electron microscopy.

Table 3. Frequencies of transposition and cointegrate formation between pBR322::Tn*5*-KS and pOX38

	Frequency per cell per generation	
Donor strain	Transposition	Cointegrate formation
LC245 (*recA*$^+$)	3.5×10^{-8}	1.5×10^{-7}
GK30 (*recA*$^-$)	4.8×10^{-8}	2.1×10^{-9}
Ratio (*recA*$^+$/*recA*$^-$)	0.73	71

The measurements (10 matings) were performed as described in *Materials and Methods*.

Table 4. Fluctuation test measurements of cointegrate formation between pBR322::Tn*5*-KS and pOX38

		Fraction of total cultures		Frequency, per cell per generation	
Donor strain	Cells $\times 10^{-6}$ per culture	No Nal^R, Km^R, Sm^R cell	No Nal^R, Km^R, Sm^R, Tc^R cell	Transposition and cointegrate formation	Cointegrate formation
LC245 (*recA*$^+$)	4.8	12/49	19/49	2.0×10^{-7}	(7.2×10^{-8})
GK30 (*recA*$^-$)	600	0	24/50	1.3×10^{-7}	9.0×10^{-10}

The number in parentheses is the transposition frequency estimated from the colony counts in the same experiment.

DISCUSSION

The results presented here demonstrate that the frequency of cointegrate formation mediated by Tn5 is comparable to that of transposition only in a recombination-proficient (*recA*$^+$) host. In a *recA*$^-$ host the tranposition frequency remains unchanged and cointegrate formation is depressed to less than 1/100.

In the case of the 5-base-pair repeating element Tn3, the *recA* system plays a role in the transposition process only if the site-specific recombination system has been inactivated. Its role, however, is in the breakdown of the cointegrates by homologous recombination between the two directly repeated elements rather than in cointegrate formation. Cointegrates are formed equally well by Tn3 in the presence or absence of *recA* function but are stable only in its absence (8). Our experiments with the 9-base-pair repeating element Tn9 (IS*1*) have shown that in the absence of *recA* function this element is capable of forming cointegrates at a frequency comparable to that of transposition (not more than 10-fold less) rather than the 100-fold difference we observe with Tn5 (11). We think it likely, therefore, that Tn5 is somewhat different in its transposition properties from Tn9, even though the cointegrates it forms are as stable as those formed by Tn9/IS*1* (13).

There are several conceivable ways in which homologous recombination functions could exert an effect on cointegrate formation. The formation of the cointegrate could be a secondary event in which the original copy of Tn5-KS, on pBR322, recombines, using the homologous recombination system, with a copy of the element that has been tranposed onto pOX38. The results of Isberg and Syvanen (12) and of our previous study (13) in which we used a pOX38::Tn5 derivative to mediate formation of cointegrate molecules with pBR322 argue against the possibility, however. Of the 23 independent cointegrates we analyzed (formed in a *recA*$^+$ background) 9 were mediated by IS*50*-R and none by IS*50*-L—i.e., this element was never found as the junction element in a cointegrate. In this "post-transposition-recombination" hypothesis, transposition of one of the two IS*50* elements to pBR322 would be expected to occur, followed by recombination with *either* of the two elements carried by pOX38::Tn5. Because cointegrates formed by homologous recombination with a transposed element should have no preference for one copy of IS*50* or the other (they differ only by one

or two base pairs in 1.5 kb) it would be very unlikely for the recombination to occur with IS*50*-R in each of the nine examples. Furthermore, the frequency of cointegrate formation in a *recA*$^+$ strain was found to be comparable to the frequency of transposition. This means that the homologous recombination between elements on separate plasmids would have to occur for almost all transposition events. We conclude that secondary homologous recombination events between completed transposition products cannot explain our results.

The data presented here appear to rule out the possibility that Tn5 transposes through a cointegrate intermediate pathway as proposed in some models of transposition (8, 31). This is because the frequency of transposition is unchanged when the *recA* background changes, whereas the cointegrate frequency changes dramatically. If the cointegrates were intermediates, this would mean that they must be breaking down at a much higher rate in the *recA*$^-$ strains (about 100-fold), which they are known not to do (unpublished). We do not favor the hypothesis that the cointegrates arise by a direct transposition event of one copy of the plasmid from a dimer, but our data do not rule out the possibility.

If the cointegrates are the products of a pathway that can yield direct transpositions (6, 14) there are several ways that the *recA* function could exert an effect. One of these is that *recA* gene product interacts in some unknown fashion with the transposition apparatus to increase the fraction of products that are cointegrates. At present there is no precedent for this kind of an effect and no known way to test the hypothesis. A more attractive possibility is that the *recA* function exerts its effect during the process of transposition. Assume for the moment that the process of transposition for Tn5 always leads to a direct transposition of the element, as described by one branch of the model proposed in ref. 6. Because during transposition the two copies of the element must be in close proximity, the *recA* system could mediate recombination between the two transposons during or just after the transposition replication step. At this step the elements may present a particularly good substrate for the *recA* system, being partially single stranded. If the normal process (unrecombined elements) yields a direct transposition, the altered process would yield a cointegrate. While we favor this hypothesis, we emphasize that it is far from proven. Alternatively, it is possible that the *recA* system is involved in the repair or rescue of aborted or damaged transposition complexes in such a way as to increase the fraction of completed cointegrates or that the mediation is indirect through the participation of *recA* in the induction of SOS-like functions.

In a recent modification of his model Shapiro has proposed that direct transposition could occur by a recombination event during or just after replication (32). Our results suggest that for Tn5 the mechanism is just the opposite: cointegrate formation rather than resolution may require a recombination event.

There are also several possible mechanisms that might explain the formation of the rare cointegrates in the absence of *recA* function. They could represent the normal products of the cointegrate formation branch of the transposition pathway proposed in the models of Harshey and Bukhari (14) and Galas and Chandler (6), or they could be the result of some residual recombination function in the *recA*$^-$ strain. An alternative and intriguing hypothesis is that they result from transposition replication that fails to terminate at the distal end of the element and continues all the way around pBR322::Tn5-KS and back into the element. In such a case, the direct transposition pathway yields a cointegrate structure. This possibility has been suggested by Grindley and Sherratt (33) and Harshey and Bukhari (14) and is implicit in our model (6).

We are grateful to Otto Jenni for his skillful preparation of the figures. This work was supported by grants from the Swiss National Fund to L. Caro (3.591.79) and J. Miller (3.493.79).

1. Starlinger, P. (1980) *Plasmid* **3**, 241–259.
2. Calos, M. & Miller, J. H. (1980) *Cell* **20**, 579–595.
3. Berg, D. E. (1977) in *DNA Insertion Elements, Plasmids and Episomes*, eds. Bukhari, A. I., Shapiro, J. & Adhya, S. L. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 205–212.
4. Kleckner, N., Barker, D., Ross, D. & Botstein, D. (1978) *Genetics* **90**, 427–450.
5. Young, R., Smith-Grillo, D., Isberg, R., Way, J. & Syvanen, M. (1980) *Mol. Gen. Genet.* **178**, 681–689.
6. Galas, D. J. & Chandler, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4858–4862.
7. Gill, R., Heffron, F., Dougan, G. & Falkow, S. (1978) *J. Bacteriol.* **136**, 742–756.
8. Arthur, A. & Sheratt, D. (1977) *Mol. Gen. Genet.* **175**, 267–274.
9. Kitts, P., Symington, L., Burke, M., Reed, R. & Sheratt, D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 46–50.
10. Heffron, F., Kostriken, R., Morita, C. & Parker, R. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 259–268.
11. Galas, D. J. & Chandler, M. (1982) *J. Mol. Biol.* **154**, 245–272.
12. Isberg, R. R. & Syvanen, M. (1981) *J. Mol. Biol.* **150**, 15–32.
13. Hirschel, B. J., Galas, D. J. & Chandler, M. (1982) *J. Mol. Biol.*, in press.
14. Harshey, R. M & Bukhari, A. I. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1090–1094.
15. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Clewell, D. B. & Helinski, D. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1159–1166.
17. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523.
18. Berg, D. E., Egner, C., Hirschel, B. J. & Howard, J. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 115–123.
19. Lane, D. & Gardner, R. C. (1979) *J. Bacteriol.* **139**, 141–151.
20. Lane, D. & Chandler, R. C. (1977) *Mol. Gen. Genet.* **157**, 17–23.
21. Luria, S. & Delbrück, M. (1943) *Genetics* **28**, 491–502.
22. Berg, D. E., Davies, J., Allet, B. & Rochaix, J. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3628–3632.
23. Jorgenson, R. A., Rothstein, S. J. & Reznikoff, W. S. (1980) *Mol. Gen. Genet.* **177**, 65–72.
24. Rothstein, S. J. & Reznikoff, W. S. (1981) *Cell* **23**, 191–199.
25. Sutcliffe, J. G. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 77–90.
26. Guyer, M. S., Reed, R. R., Steitz, J. A. & Low, K. B. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 135–140.
27. Guyer, M. (1978) *J. Mol. Biol.* **126**, 347–365.
28. Grindley, N. D. & Joyce, C. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7176–7180.
29. Hayes, W. (1968) *The Genetics of Bacteria and Their Viruses* (Wiley, New York), 2nd Ed., p. 195.
30. Johnson, D. A. & Willetts, N. (1980) *Plasmid* **4**, 292–304.
31. Shapiro, J. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1933–1937.
32. Shapiro, J. (1980) in *Plasmid and Transposons*: Environmental Effects and Maintenance Mechanisms, eds. Stuttgard, C. & Rozee, K. R. (Academic, New York), pp. 229–247.
33. Grindley, N. D. & Sheratt, D. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1257–1261.