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Genetics

Excision of transposon Tn*5* is dependent on the inverted repeats but not on the transposase function of Tn*5*

(antibiotic resistance/homologous recombination/reversion/*lac* operon)

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ABSTRACT **The excision of Tn*5* from sites of insertion in the *Escherichia coli* genome was studied by examining the reversion of *lac*::Tn*5* insertion mutations to *lac*$^+$. We find that: (*i*) the frequency of excision depends on the site of Tn*5* insertion, (*ii*) excision occurs efficiently in *recA*$^-$ cells, (*iii*) excision does not require a Tn*5*-encoded transposition function, and (*iv*) efficient excision requires the inverted repeats of Tn*5*. We propose that excision of Tn*5* is similar to the formation of spontaneous deletions and occurs by slippage during DNA synthesis.**

The kanamycin resistance (Kan^r) determinant Tn*5* (1–3) is representative of a class of prokaryotic DNA segments known as transposons which have the ability to move from site to site in a genome independent of extensive DNA sequence homology (for review see refs. 4 and 5). Transposition is thought to depend on recognition of specific sequences near the ends of the transposon by an enzyme termed a transposase. Tn*5* and several other transposons are known to encode proteins necessary for their own transposition (6–8).

Tn*5* is 5700 base pairs (bp) long and contains 1532-bp inverted repeats that are themselves transposons now designated IS*50*-L and IS*50*-R (9) bracketing a central region containing a Kan^r determinant. The two copies of IS*50* in Tn*5* differ functionally. IS*50*-R encodes a function necessary for Tn*5* transposition. A single bp change in IS*50*-L has simultaneously created a promoter used for expression of the *kan*^{r} gene (designated *P* in Fig. 1) and an ochre mutation in IS*50*-L's transposase gene. Analysis of proteins produced in minicells containing cloned segments of Tn*5* indicated that IS*50*-R encodes two proteins (58,000 and 54,000 daltons); IS*50*-L encodes two smaller proteins (53,000 and 49,000 daltons), which are likely to be the amino-terminal ochre fragments of the proteins encoded by IS*50*-R. DNA sequence analysis revealed only one large open reading frame in IS*50* and thus it seemed likely that the pairs of proteins from each copy of IS*50* result either from initiation of translation at two different sites or from posttranslational protein processing (7, 10, 11).

Tn*5* excises by a process that does not lead to its transposition to new sites (2). Excision can be detected by reversion of insertion mutations (precise excision), or by relief of transcriptional polarity without restoration of the original gene sequence (imprecise excision). Precise excision of Tn*5* from sites in *lac* in F′ *lac* episomes occurs at an average frequency of about 10^{-6}. Imprecise excision from *lacZ* occurs at an average frequency of about 10^{-5} (ref. 2, and this work).

Because totally random events would not remove blocks of DNA the size of Tn*5* precisely, an explanation for the specificity of excision is needed. Two general types of models can be formulated: In one, the ends of Tn*5* are recognized and brought together by a specific enzyme, the ends of Tn*5* are cleaved, and the adjacent bacterial sequences are rejoined. An obvious candidate for such a specific enzyme is Tn*5*'s transposase (2), by analogy with the excision of phages λ and Mu (12, 13).

In the other model, excision of Tn*5* occurs by mechanisms involved in the formation of a class of spontaneous deletions that have end points in short, closely spaced direct repeats. This model (detailed in *Discussion*) incorporates findings that Tn*5* creates 9-bp direct duplications of target sequences at its site of insertion (14), contains long terminal inverted repeats (1), and is extremely unstable when inserted into the genome of single-stranded DNA phage fd (15).

To better understand the mechanism of excision we tested whether either transposase or the inverted orientation of Tn*5*'s repeats is necessary for excision. We show here that derivatives of Tn*5* that have lost the transposase function retain the ability to excise, whereas transposition-proficient derivatives of Tn*5* in which its component IS*50* elements are in direct rather than inverted orientation excise inefficiently. These results support a model in which excision results from copy errors during replication of DNA segments with inverted repeats.

MATERIALS AND METHODS

Recipes for broth and minimal media, and general procedures for phage growth, conjugation, and transduction have been reported elsewhere (1–3, 16). Difco Noble agar was used instead of Difco Bacto agar to select lactose-utilizing (Lac$^+$) revertants at 37°C. *lacY*$^+$ revertants were selected on melibiose Noble agar plates at 41°C (9, 16). The *Escherichia coli* strains used are listed in Table 1. *lac*::Tn*5* insertion mutants were generated by transposition of Tn*5* from phage λ *b*221 *c*I857 Tn*5* to the *lac* operon in strain DB1446 (3). The initial screening for reversion by the formation of Lac$^+$ papillae was carried out in this set of strains. *lac*::Tn*5* alleles to be examined more closely were transduced into strain DB1506 by using phage P1 Cm clr100 (3, 16).

A map of Tn*5* and the derivatives used is given in Fig. 1. λ phage strains carrying the Tn*5* derivatives shown in Fig. 1*A* were generated by homologous recombination *in vivo* between the IS*50* elements of a Tn*5* element in a λ *b*221 *c*I857 genome and a second Tn*5* element present in a plasmid (3, 20). The replacement of Tn*5* at specific sites in *lac* by the derivatives shown in Fig. 1*A* was carried out by homologous recombination between the IS*50* elements of two transposons (3, 20).

Tn*5*-DR2, which contains direct repeats of IS*50*, is shown in Fig. 1*B*. *lacZ*::Tn*5*-DR2 alleles were generated by transposition of Tn*5*-DR2 from a λ prophage to the *lac* operon in an

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Abbreviations: Kan^r, kanamycin resistance; Tet^r, tetracycline resistance; Lac$^+$, lactose utilization; Tn$^+$, transposition proficient; Tn$^-$, transposition deficient; bp, base pair(s); IS*50*-L, the left inverted repeat of TN*5*; IS*50*-R, the right inverted repeat of Tn*5*; WT, wild type.

Table 1. Bacterial strains

Strain	Genotype	Origin
DB104	F⁻ Δ(*proB–lac*) Δ*trpE5 supII*⁺ *str*ʳ (λ *b515 b519 xis* am6 cI857 *nin5* Sam 7)	This work
DB114	F⁻ Δ*trpE5 hfl-1*	This work
DB149	F⁻ Δ*trpE5* (λ *b515 b519 xis* am6 cI857 *nin5* Sam 7)	This work
DB1446	HfrC *proC met str*	Ref. 3
DB1504	F⁻ Δ(*proB–lac*) Δ*trpE5 supII*⁺	Ref. 3
DB1506	F′ *proB*⁺ *lac*⁺/Δ(*proB–lac*) Δ*trpE5 supII*⁺	Ref. 3
DB1648	F⁻ Δ(*proB–lac*) Δ*trpE5 supII*⁺ *recA str*	This work

DB104 is a *str*ʳ derivative of DB1504 lysogenized with the indicated λ phage provided by R. Weisberg. DB114 is derived by P1CM-linked cotransduction of *purA*⁺ *hfl-1* (17) into a *purA*::Tn*5* (18) derivative of DB1470 (16), the *pro*⁺ *lac*⁺ parent of DB1504. The *hfl-1* allele enhances the efficiency of lysogenization by phage λ (17). DB149 is a lysogenic derivative of DB1470. DB1648 was derived by linked cotransfer of *thyA*⁺ *recA*⁻ alleles into a *thyA*⁻ *str*ʳ derivative of DB1504.

F′*lac* episome. *lacZ*::IS*50* derivatives of *lacZ*::Tn5-DR2 alleles were generated by *recA*⁺-dependent homologous recombination between the directly repeated IS*50* sequences. DNA sequence analysis of IS*50* inserted into plasmid pBR322 revealed a short, imperfect, inverted repeat structure (7 of 8 bp) at IS*50*'s ends, and the duplication of 9 bp of target sequence at the site of insertion (9).

To measure transposition of Tn5 and its derivatives from sites in the *lac* operon, we transferred F′*lac* episomes containing Tn5 elements to lysogenic strain DB104, induced the λ prophage, and determined the frequencies of Kanʳ, Tetʳ, or tryptophan-independence transducing phage after infection of strain DB114. The data presented in *Results* are corrected for the fraction of lysogens among survivors (usually ≈0.8), and the multiplicity of infection (5 phage per cell). Transductants were selected five generations after infection because preliminary experiments, in which cultures were plated within a few generations of infection, showed considerable variability in the yields of Kanʳ and Tetʳ transductants. Rare tryptophan-independent or Kanʳ transductant colonies (frequencies of $<10^{-8}$) were obtained in some assays of transposition of Tn5-410 and Tn5-112. These transductants did not contain λ Tn5-410 or λ Tn5-112 prophages and probably arose from λ's ability to act as a generalized transducing phage (21).

RESULTS

Precise Excision of Tn5-WT. We used the occurrence of Lac⁺ revertants of *lac*::Tn5 insertion mutants to study the excision of Tn5 because excision is too rare to detect simply by screening for kanamycin-sensitive colonies. When *lac*::Tn5 mutants are grown on MacConkey agar, Lac⁺ revertants emerge as red papillae after 1–2 days of growth at 37°C. Each of 150 independent *lac*::Tn5 insertion mutants tested formed Lac⁺ revertant papillae.

The number of Lac⁺ revertant papillae per colony, which reflects the rate of reversion, differs markedly among independent insertion mutants (from less than 1 to more than 10 per colony after 2–3 days of incubation at 37°C). When cultures were plated on minimal lactose agar, the average Lac⁺ revertant

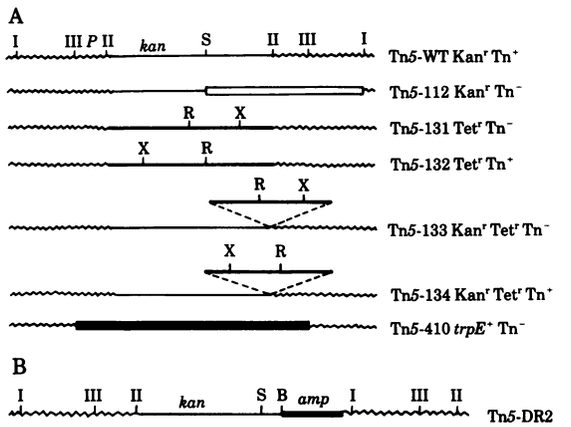

FIG. 1. Map of Tn*5* and its derivatives (adapted from refs. 6, 9, 10, 11, and 19). Jagged lines indicate the 1532-bp inverted terminal repetitions of Tn*5* which are themselves transposons termed IS*50*-L (left) and IS*50*-R (right). *P* indicates the promoter for expression of Kanʳ, the result of a single bp change 1442 bp from the outside end of IS*50*-L. This change also creates an ochre mutation in IS*50*-L's transposase gene. Restriction endonuclease cleavage sites: I, *Hpa* I at 197 bp; II, *Bgl* II at 1515 bp; III, *Hin*dIII at 1195 bp; R, *Eco*RI; X, *Xba* I; B, *Bam*HI. Tn⁺ and Tn⁻ indicate transposition-proficient and transposition-deficient, as defined in the text. A likely reading frame for transposase extends from 92 bp to 1520 bp in IS*50*-R. Transposition of IS*50*, like transposition of Tn*5*, causes 9-bp direct duplications of target sequences. The ends of IS*50* are themselves short imperfect (7 of 8 bp) inverted repetitions. (*A*) Substitution and deletion derivatives of Tn*5*. Tn*5*-131 and Tn*5*-132 contain a 2700-bp fragment from Tn*10* encoding tetracycline resistance (Tetʳ) as a substitution in place of the central *Bgl* II fragment of wild-type Tn*5* (Tn*5*-WT). In Tn*5*-133 and Tn*5*-134, the same *Bgl* II Tetʳ fragment is inserted into the Tn*5* element in the right component of the inverted repetition. Tn*5*-410 contains the *trp* operon *E* and *D* genes in place of the central *Hin*dIII fragment of wild-type Tn*5*. (*B*) A Tn*5* derivative with direct repeats, Tn*5*-DR2, was generated by ligation after *Bam*HI digestion of a pBR322::Tn*5* plasmid. Both the *ampʳ* gene of pBR322 and the *kanʳ* gene of Tn*5* are present between the direct repeats of IS*50*.

Table 2. Excision frequencies of Tn*5* derivatives

Insertion site	Lac⁺ revertant frequencies × 10^6													
	Tn*5*-WT (Tn⁺)		Tn*5*-131 (Tn⁻)		Tn*5*-132 (Tn⁺)		Tn*5*-133 (Tn⁻)		Tn*5*-134 (Tn⁺)		Tn*5*-410 (Tn⁻)		TN*5*-112 (Tn⁻)	
Z155	0.20 ± 0.10	(10)	0.083 ± 0.009	(5)	0.090 ± 0.010	(5)	0.039 ± 0.009	(5)	0.051 ± 0.022	(9)	0.052 ± 0.009	(5)	0.035 ± 0.009	(5)
Z217	1.9 ± 0.3	(5)	1.7 ± 0.2	(5)	1.4 ± 0.5	(5)	0.78 ± 0.16	(5)	0.82 ± 0.18	(5)	0.64 ± 0.30	(5)	—	
Z202	7.5 ± 1.4	(5)	3.2 ± 1.2	(10)	3.2 ± 0.6	(10)	1.3 ± 0.4	(10)	1.9 ± 0.7	(10)	1.1 ± 0.2	(5)	0.23 ± 0.08	(5)
Y148	22 ± 8	(5)	16 ± 7	(10)	16 ± 3	(10)	9.8 ± 2.6	(10)	7.5 ± 4.1	(10)	11 ± 4	(4)	2.0 ± 0.4	(5)
Y141	230 ± 30	(5)	42 ± 4	(5)	34 ± 15	(5)	9.8 ± 2.8	(5)	6.8 ± 1.8	(5)	—		—	

This table reports mean frequencies of Lac⁺ revertants ± the standard deviations obtained after approximately 34 generations of clonal growth from single cells. The numbers of cultures examined for each determination are given in parentheses. Cultures of the DB1506 derivatives containing the indicated *lac*::Tn*5* insertion mutations were streaked on broth agar without antibiotics and incubated at 37°C for 12 hr. Individual colonies were suspended in 11 ml of broth and shaken at 37°C for 10 hr. The stationary-phase cultures (density of 1–2 × 10^9 cells per ml) were washed once with 10 mM $MgSO_4$ and resuspended in 10 mM $MgSO_4$. The optical density of each suspension was determined to estimate cell titer (OD_{600} = 1 corresponds to 7 × 10^8 cells per ml) and samples expected from preliminary experiments to contain 50–200 Lac⁺ revertants were then spread on each of two minimal lactose plates and incubated at 37°C for 36 hr.

frequency was 10^{-6}, with a range from 3×10^{-8} to 2×10^{-4} among 10 different Tn5 insertion mutants in otherwise isogenic F'*lac*::Tn5 strains (column 2 of Table 2, and unpublished data). Upon transfer of representative F'*lac*::Tn5 episomes into *recA*$^-$ strain DB1648, the Lac$^+$ revertant frequencies were decreased severalfold. Cultures of *recA*$^-$ strains grow poorly, however. Consequently, the differences we observed could reflect either inefficient recovery of revertants in *recA*$^-$ cells or a direct, but minor, role of *recA* in Tn5 excision.

We also transduced representative *lac*::Tn5 alleles into the chromosomes of F$^-$ strains and found that they revert at frequencies approximately 1/100th of those for the same alleles in F'*lac* episomes. Hopkins and coworkers (22) have made similar observations and identified genes in F involved in enhanced excision.

Derivatives of Tn5 Deficient in Transposition. To assess the possible role of transposase in Tn5 excision, we examined the excision of the set of Tn5 derivatives diagrammed in Fig. 1*A*. Because the revertant frequency depends on the site of insertion, we generated sets of isogenic strains that differed only in the particular Tn5 element present at a given site in *lac*. Proof that the replacement of one Tn5 element by another did not alter the transposition proficiency was obtained by measuring the frequency of transposition of the element from an F'*lac* episome to phage λ. The following average frequencies were obtained: Tn5-WT, 5×10^{-6}; Tn5-132, 4×10^{-6}; Tn5-134, 8×10^{-6}; Tn5-131, 2×10^{-8}; Tn5-133, 1×10^{-8}; Tn5-112 and Tn5-410, less than 10^{-9}; Tn5-DR2, 2×10^{-6}; Tn5-WT from sites in *lac* in F$^-$ strain DB149, 5×10^{-6}. This last result shows that F has no significant effect on the frequency of transposition even though it enhances excision of Tn5.

Because functional tests and DNA sequence analyses indicate that only IS*50*-R contains a functional transposase gene and that the coding sequence of this gene terminates 5 bp after the *Bgl* II site in IS*50*-R (7, 10, 11), the dependence of transposition frequency on the orientation of an inserted *tet*^{r} segment suggests that an altered transposase encoded by Tn5-131 and Tn5-133 retains less activity than that encoded by Tn5-132 and Tn5-134.

Excision Independent of Transposition Proficiency. The results of reversion tests (Table 2) show that the Tn$^-$ Tn5 elements can undergo excision. We find no differences in the Lac$^+$ reversion frequencies of mutations in which structurally similar Tn5 elements are either proficient or deficient in transposition: e.g., Tn5-132 vs. Tn5-131 and Tn5-134 vs. Tn5-133 (Fig. 1). The differences in frequencies of excision from any given site that we do detect may be effects of the size or sequence organization of the inserted element. The efficient excision of Tn$^-$ derivatives of Tn5 rules out excision models dependent on the action of a Tn5-encoded transposase.

Table 3. *lacY*$^+$ revertants of *lacZ* insertion mutant *lacZ*217::Tn5

Tn5 element	*lacZ*$^+$	*lacZ*$^-$ Revertible	*lacZ*$^-$ Nonrevertible	Total
Tn5-WT	59	31	12	102
Tn5-131 (Tn$^-$)	80	16	5	101
Tn5-132 (Tn$^+$)	82	15	4	101

lacY$^+$ revertants were selected by spreading young broth cultures on minimal melibiose agar plates and incubating them for 2 days at 41°C. One *lacY*$^+$ revertant colony from each streak was restreaked for purification on MacConkey melibiose agar. Single *lacY*$^+$ colonies were picked and tested on MacConkey lactose agar to determine if they were *lacZ*$^+$ or *lacZ*$^-$. *lacZ*$^-$ clones were restreaked on MacConkey agar and incubated 2 days at 37°C to distinguish the revertible and nonrevertible *lacZ*$^-$ alleles by their ability to form Lac$^+$ papillae.

Precise Excision of Tn5-DR2, a Tn5 Derivative with Direct Terminal Repeats. We assessed the role of the orientation of Tn5's IS*50* elements by using 18 independent Lac$^-$ mutants generated by insertion of Tn5-DR2. Each gave rise to a very small number of Lac$^+$ revertant papillae (less than 1 per 20 colonies) after prolonged incubation on MacConkey agar. The average frequencies of Lac$^+$ revertants in cultures of five representative *lac*::Tn5-DR2 mutants on minimal lactose agar was 3×10^{-9}. These results contrast with the average Lac$^+$ revertant frequency of 10^{-6} for *lacZ*::Tn5-WT insertion mutants.

Precise Excision of IS*50*, One Inverted Repeat of Tn5. We measured the reversion of *lacZ*::IS*50* derivatives of the five *lacZ*::Tn5-DR2 mutants. On MacConkey agar Lac$^+$ papillae were very rare. On minimal lactose agar the average Lac$^+$ revertant frequency was 8×10^{-9} (as compared with 2×10^{-9} for the parental *lacZ*::Tn5-DR2 mutants). Thus, although derivatives of Tn5 that lack long inverted repeats can be excised at low frequency, it is clear that efficient precise excision depends on the inverted repeat sequence organization of wild-type Tn5.

Imprecise Excision. The *lacZ* gene is transcribed before *lacY*, and insertions of Tn5 and of Tn5-DR2 in *lacZ* result in a loss of *lacY* expression (3). Three classes of *lacY*$^+$ revertants of *lacZ*::Tn5 mutants are generally found: (*i*) *lacZ*$^+$ true revertants, (*ii*) *lacZ*$^-$ that can revert to *lacZ*$^+$ and probably retain part of Tn5, and (*iii*) *lacZ*$^-$ that never revert to *lacZ*$^+$ and are usually deletions of part of *lacZ* (2, 3). Table 3 shows that imprecise excision is independent of whether the excised Tn5 element is Tn$^+$ or Tn$^-$.

To compare the imprecise excision of Tn5-DR2 and Tn5-WT, we transferred 18 *lacZ*::Tn5-DR2 mutants and 10 *lacZ*::Tn5-WT mutants present in F' episomes into *recA*$^-$ strain DB1648 and measured the frequencies of *lacY*$^+$ revertants (a *recA*$^-$ strain was used to prevent the breakdown of Tn5-DR2 to IS*50* by homologous recombination). The average frequency of *lacY*$^+$ revertant colonies was 10^{-6} for Tn5-WT and 2×10^{-8} for Tn5-DR2 mutants. Of 87 *lacY*$^+$ derivatives of the *lacZ*::Tn5-DR2 mutants tested, none was *lacZ*$^+$. Thus, terminal inverted repetitions facilitate imprecise as well as precise excision of Tn5 elements.

DISCUSSION

Our experiments indicate that excision of Tn5 occurs by a process that is different from transposition to new sites. Although both events are largely *recA* independent: (*i*) excision of Tn5 from one site is not correlated with its movement to new sites; (*ii*) the frequency of excision, unlike that of transposition, depends on the site of Tn5 insertion and is strongly enhanced when Tn5 is inserted into F' episomes or Hfr chromosomes; (*iii*) derivatives of Tn5 that cannot transpose still excise efficiently; (*iv*) Tn5-DR2, a Tn5 derivative with direct instead of inverted terminal repeats, transposes normally but is deficient in excision.

These results argue against models in which there is an enzyme common to excision and transposition. Comparisons between structurally similar elements that differ in their ability to transpose (Tn5-131 vs. Tn5-132 and Tn5-133 vs. Tn5-134) are most relevant because, although the excision frequencies are somewhat different from that of Tn5-WT, excision is not affected by the ability of these elements to transpose. The inefficient excision of Tn5-DR2 is important, because it eliminates the possibility that Tn5-131, Tn5-133, and Tn5-410 encode amino-terminal fragments of transposase that catalyze excision, but not transposition. Because Tn5-DR2 encodes the entire transposase but excises poorly, the ability to transpose is not sufficient for excision. Although we cannot rule out models

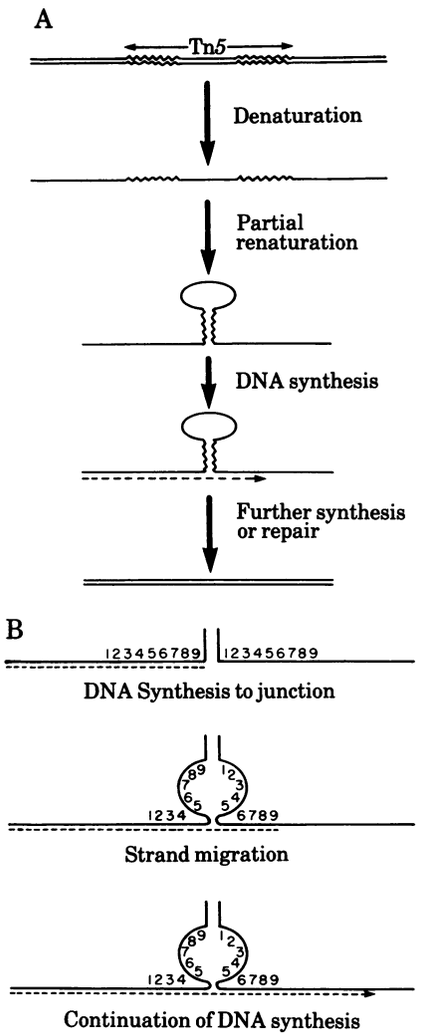

FIG. 2. Model for excision. (*A*) The way in which the inverted repeats of transposons such as Tn*5* may facilitate precise excision. (*B*) Branch migration (slippage) between direct repeats that have been juxtaposed by denaturation and partial renaturation as in *A* can lead to precise excision. Numbers 1–9 indicate the 9 bp of target DNA sequence duplicated by Tn*5* insertion. The broken line represents newly synthesized DNA and contains the nucleotide sequence complementary to 1–9. The vertical lines represent the ends of the Tn*5* transposon.

based on recognition of special extended sequences present only at the outside ends of IS*50*, an explanation in which Tn5 excision is facilitated by its inverted repeats independent of its special status as a transposon appears more likely at this time. Our proposal (Fig. 2) is therefore an attempt to show how interactions between inverted repeats of transposons such as Tn5 could bring the 9-bp direct repeats of flanking host sequence into juxtaposition and permit the rare, but normal, process of spontaneous deletion formation to operate.

We envision (*i*) that double-stranded DNA can be rendered single stranded, (*ii*) that complementary sequences corresponding to the inverted repeats in a single strand will occasionally form double-stranded segments (Fig. 2*A*), and (*iii*) that preferential use of single-stranded DNA as a template in DNA synthesis will favor the slippage of the end of the nascent chain between the first and second copy of the 9-base repeat, continuation of synthesis, and thus excision of the intervening DNA (Fig. 2*B*). Slippage between repeated sequences has been postulated previously to account for frameshifts in phage T4 (23) and specific deletion end points in *lacI* (24, 25). Our model is supported by (*i*) the instability of Tn5 inserted into the genome of single-stranded DNA phage fd during growth *in vivo* (15) and during DNA replication *in vitro* (ref. 26; K. Neugebauer and H. Schaller, personal communication), (*ii*) the excision deficiency of Tn5-DR2 and IS*50* (this work), (*iii*) the ability of the *recBC* and *rep* proteins to cause extensive denaturation of DNA *in vitro*, provided that excess single-stranded DNA binding protein is present (27, 28), and (*iv*) the preferential use of single-stranded DNA templates by DNA polymerase I and its ability to switch templates *in vitro* (29, 30).

Because imprecise excision is also stimulated by inverted repeats, and is independent of the transposase function, imprecise excision may occur by similar processes. Kleckner and colleagues (31) found that short inverted repeat sequences present within the long terminal inverted repeats of Tn*10* (Tet^r) serve as hot spots for the imprecise excision of that element, and they independently suggested that stem and loop structures similar to those in Fig. 2*A* might be involved in imprecise excision. Thus, we envision that slippage of the nascent strand between short direct repeats other than those that immediately bracket Tn5 could cause loss of host sequences and generate the nonrevertible $lacZ^-$ strains in Table 3. Template switching—a copying of one strand of the stem and then reversal to copy the complementary strand, essentially as proposed for the formation of λ*dv* plasmids (32) and as seen in ColE1 plasmid replication (33)—would cause loss of the central region of Tn5 and generate the revertible $lacZ^-$ strains seen in Table 3.

We speculate that any DNA segment containing inverted repeats can be excised by copy errors after denaturation and intramolecular renaturation as outlined here. This might even include sequences such as IS*1* and Tn*3* if their short (less than 40-bp) terminal repeats (4) are sufficiently stable. Analogous excision mechanisms may operate in eukaryotes, because certain segments excised during the differentiation of the immune system contain terminal inverted repeats (34). Programmed excision of these antibody gene segments might be independent of DNA cleavage, occurring instead by a developmentally regulated local strand separation, formation of a new segment of double-stranded DNA, and DNA synthesis.

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1. Berg, D. E., Davies, J., Allet, B. & Rochaix, J. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3628–3632.
2. Berg, D. E. (1977) in *DNA Insertion Elements, Plasmids and Episomes*, eds. Bukhari, A. I., Shapiro, J. A. & Adhya, S. L. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 205–212.
3. Berg, D. E., Weiss, A. & Crossland, L. (1980) *J. Bacteriol.* **142**, 439–446.
4. Calos, M. & Miller, J. H. (1980) *Cell* **20**, 579–595.
5. Starlinger, P. (1980) *Plasmid* **3**, 241–259.
6. Meyer, R., Boch, G. & Shapiro, J. A. (1979) *Mol. Gen. Genet.* **171**, 7–13.
7. Rothstein, S., Jorgensen, R., Postle, K. & Reznikoff, W. (1980) *Cell* **19**, 795–805.
8. Gill, R. E., Heffron, F. & Falkow, S. (1979) *Nature (London)* **282**, 797–801.
9. Berg, D. E., Egner, C., Hirschel, B. J., Howard, J., Johnsrud, L., Jorgensen, R. A. & Tlsty, T. D. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, in press.
10. Auerswald, E.-A., Ludwig, G. & Schaller, H. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, in press.
11. Rothstein, S. J., Jorgensen, R. A., Yin, J. C.-P., Yong-di, Z., Johnson, R. C. & Reznikoff, W. S. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, in press.
12. Enquist, L. W. & Weisberg, R. W. (1977) *J. Mol. Biol.* **111**, 97–120.
13. Bukhari, A. (1975) *J. Mol. Biol.* **96**, 87–99.

14. Schaller, H. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 401–408.
15. Hermann, R., Neugelbauer, K., Zentgraf, H. & Schaller, H. (1978) *Mol. Gen. Genet.* **159**, 171–178.
16. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Belfort, M. & Wulff, D. (1973) *J. Bacteriol.* **115**, 299–306.
18. Shaw, K. J. & Berg, C. M. (1979) *Genetics* **92**, 741–747.
19. Jorgensen, R. A. (1978) Dissertation (Univ. Wisconsin, Madison, WI).
20. Berg, D. E., (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4880–4884.
21. Sternberg, N. & Weisberg, R. W. (1975) *Nature (London)* **256**, 97–103.
22. Hopkins, J. D., Clements, M. B., Liang, T. S., Isberg, R. R. & Syvanen, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2814–2828.
23. Okada, Y., Streisinger, G., Emrich, J., Newton, J. Tsugita, A. & Inouye, M. (1972) *Nature (London)* **236**, 338–341.
24. Farabaugh, P. I., Schmeissner, U., Hofer, M. & Miller, J. H. (1978) *J. Mol. Biol.* **126**, 847–863.
25. Galas, D. (1978) *J. Mol. Biol.* **126**, 558–563.
26. Neugebauer, K., Zentgraf, H., Herrmann, R. & Schaller, H. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358**, 282 (abstr.).
27. Rosamond, J., Telander, K. M. & Linn, S. (1979) *J. Biol. Chem.* **254**, 8646–8652.
28. Yarronton, G. T. & Gefter, M. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1658–1652.
29. Richardson, C., Inman, R. B. & Kornberg, A. (1964) *J. Mol. Biol.* **9**, 46–69.
30. Masamune, Y. & Richardson, C. C. (1971) *J. Biol. Chem.* **246**, 2692–2701.
31. Ross, D. G., Swan, J. & Kleckner, N. (1979) *Cell* **16**, 733–738.
32. Chow, L., Davidson, N. & Berg, D. (1974) *J. Mol. Biol.* **86**, 69–89.
33. Bachmann, K., Betlach, M., Boyer, H. W. & Yanofsky, S. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 69–76.
34. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. (1980) *Cell* **19**, 981–992.