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# THE MECHANISM AND CONTROL OF Tn*5* TRANSPOSITION

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## SUMMARY

*Analyses of transposition from pBR322::Tn5 plasmids to the F factor of E. coli show that Tn5 and its component IS50 elements do not generate cointegrates and suggest that IS50-mediated transposition is conservative. Our experiments also indicate that: (i) The Tn5 encoded transposase acts to promote transposition in cis, but not in trans. (ii) A product of the Tn5 transposase gene also inhibits Tn5 transposition; this inhibition operates in trans. (iii) Transcription near Tn5 in the donor DNA molecule directly inhibits transposition. (iv) Transcription of the target DNA molecule appears to stimulate transposition.*

## INTRODUCTION

Transposable elements are specialized DNA segments which move from site to site in a genome, independent of the extensive DNA sequence homology necessary for classical recombination. These ubiquitous elements have attracted much attention (i) as frequent causes of spontaneous mutation and genome rearrangement, (ii) as developmentally regulated modulators of gene expression, and possible instigators of developmental diseases such as cancer, (iii) as agents which speed the flow of genes conferring antibiotic resistance and pathogenicity between different bacterial species, (iv) as powerful new tools for incisive molecular and genetic analyses, and (v) because of intrinsic interest in the types of DNA joining mechanisms which must be involved in their movement. Among transposable elements are McClintock's mobile controller elements in maize, the retroviruses of vertebrates, the transcribed middle repetitive sequences such as copia and Ty-1 of *Drosophila* and yeast, and the simple IS elements, the antibiotic resistance transposons, and some of the temperate phage of bacteria. Most transposable elements generate short direct duplications of target sequences

upon transposition. Among prokaryotic elements, groups which make 4 bp (IS*5*), 5 bp, (IS*2*, Mu, Tn*3*, γδ, Tn*501*, Tn*1721*) 9 bp (Tn*5*, Tn*9*, Tn*10*, Tn*903*) and 11 or 12 bp (IS*4*) duplications have been found (for reviews, BERG & BERG 1981; KLECKNER 1981; CALOS & MILLER 1980; STARLINGER 1980; CAMPBELL et al. 1979; FINCHAM & SASTRY 1974; Cold Spring Harbor Symposium 1980).

Transposon Tn*5* ($Kan^r$) (Fig. 1), a member of the class of elements which makes 9 bp target sequence duplications, is a composite containing terminal inverted repeats of the 1533 bp insertion sequences named IS*50*R (right) and IS*50*L (left) (Fig. 1; BERG et al. 1980a, 1982; HIRSCHEL & BERG 1982; ISBERG & SYVANEN 1981). It is not homologous to sequences in the chromosome of *Escherichia coli* K12 (BERG & DRUMMOND 1982). IS*50*R contains a transposase gene which encodes two proteins 421 and 461 amino acids long from a pair of translation initiation sites 120 bases apart on the same mRNA. The larger protein is essential for transposition. IS*50*L differs from IS*50*R in a single base pair 92 nucleotides from its inside end, and because of this difference contains a mutant allele of the transposase gene and the promoter used for expression of the centrally located kanamycin resistance gene (ROTHSTEIN et al. 1980; ROTHSTEIN & REZNIKOFF 1981; JOHNSON & REZNIKOFF 1981; AUERSWALD et al. 1980).

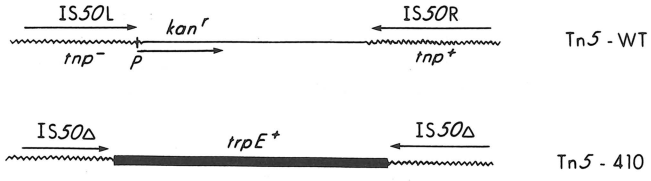


Figure 1. Maps of Tn*5*-wild type (WT) AND Tn*5*-410. Tn*5*-WT is 5700 bp long, and contains inverted repeats of the 1533 bp long insertion sequence IS*50* (represented by jagged lines) bracketing a central region containing a $kan^r$ gene (BERG et al. 1982: HIRSCHEL & BERG 1982; AUERSWALD et al. 1980). Tn*5*-410 was derived rom Tn*5*-WT by in vitro replacement of Tn*5*'s central 3.3 kb HindIII fragment with a 6 kb HindIII fragment containing the $trpPOE^+D^+$ region (MEYER et al. 1979). The internal 340 bp of each IS*50* element are missing in Tn*5*-410; these deletions are indicated by Δ. The placement of Tn*5*-410 at sites previously defined by Tn*5*-WT insertions by homologous recombination has been described (BERG 1980).

Transposase proteins such as those encoded by IS*50*R are thought to recognize distinctive sequences at the ends of the transposable elements which encode them, and thereby to confer specificity in the transposition process. DNA sequence analysis has established that the termini of IS*50* consist of a hyphenated 8 of 9 bp inverted repeat (BERG et al. 1982). This

repeat is shorter than repeats at the termini of most other IS elements. Functional tests which rely on the ability of IS*50* elements to mediate the transposition of any DNA segment they bracket have indicated that the inside ends of IS*50* are less active than the outside ends. This result suggested that the 8 bp common to both ends constitutes the core of a longer optimal transposase recognition site which is present only at the outside end of IS*50* (SASAKAWA & BERG 1982).

The experiments presented here were designed to test whether Tn*5* transposes by a conservative or a replicative mechanism, and to give insights into how Tn*5* transposition is controlled.

## RESULTS

### MODELS OF TRANSPOSITION

There are two classes of transposition models, conservative and replicative. In the conservative model (BERG 1977), an element is cleaved from its vector replicon and inserted without replicating into a target DNA molecule. The remaining segment of the donor is destroyed (Fig. 2). This model was suggested by findings that (i) the excision of Tn*5* monitored by reversion of Tn*5* induced insertion mutations was not correlated with its movement to new sites, and (ii) most clones in which Tn*5* had transposed to the *E. coli* chromosome following infection was a λ::Tn*5* phage under conditions in which lysogens were viable were not lysogenic for the λ vector (BERG 1977). Precedent for conservative insertion is provided by phages λ and Mu (NASH 1981; LIEBHART et al. 1982).

Models in which transposition is replicative include both bidirectional (SHAPIRO 1979; ARTHUR & SHERRATT 1979) and unidirectional rolling circle (BUKHARI 1981; GALAS & CHANDLER 1981) schemes. In bidirectional models nicks are introduced into complementary strands at the two ends of the element and free ends are joined to the target thereby creating forks from which replication is initiated. Replication of the element from these forks generates a cointegrate in which donor and target molecules are joined by direct repeats of the transposed element. These models were suggested by findings of cointegrates after transposition of induced Mu prophages (CHACONAS et al. 1980), and of elements related to Tn*3*. The detection of cointegrates mediated by members of the Tn*3* family was complicated in early experiments by the action of resolvase proteins which mediate crossingover between direct repeats of members of this family and thereby breakdown cointegrates into separate donor and target replicons, each with a copy of the transposable element (REED 1981; KOSTRIKEN et al. 1981; KITTS et al. 1982). Failure to detect cointegrates following transposition of Tn*5* has been attributed to the action of a Tn*5*-specific resolvase (MEYER et al. (1979).

In rolling circle models the joining of an end of one strand of an element to its target creates a fork from which replication of the element proceeds unidirectionally. Depending

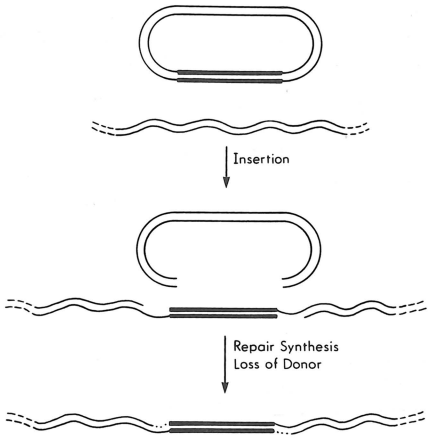


Figure 2. The conservative model for transposition. The donor molecule, the transposable element, and part of the target DNA molecule are represented by the elliptical structure, the thickened lines and the wavy lines, respectively. Transposition proceeds via the recognition and cleavage at termini of the element, and (in the case of IS*50* and Tn*5*) cleavage of the two strands of target DNA staggered by 9 bp, insertion of the element between the termini of the target, repair synthesis (indicated by dots) to fill the gaps, and ligation.

on how DNA strans are joined when transposition terminates, either cointegrates or separate donor and target replicons, each with a copy of the element, are generated. These models were developed in the belief that insertion of all nonlambdoid elements must be replicative, and to avoid invoking resolution in explaining the failure to obtain cointegrates after Mu infection and in some transpositions of Tn*9* (HARSHEY et al. 1982); GALAS & CHANDLER 1982). Direct visualization of DNAs from induced lysogens that contain Mu replicating from just one end provided support for a rolling circle model (HARSHEY et al., 1982). However, the recent demonstration that insertion of Mu DNA into target DNAs during infection is conservative (LIEBHART et al. 1982) emphasizes that failures to obtain cointegrates need not be attributed to replicative transposition coupled with a specific pattern of strand joining during termination, nor to the resolution of short-lived cointegrates.

In recent years many authors have accepted the notion that all bona fide transposable elements move by a replicative process (e.g., CALOS & MILLER 1980; KLECKNER 1981; CAMPBELL 1980; BUKHARI 1981; GALAS & CHANDLER 1981). This may be based on a feeling that explanations of the behavior of Tn*3*, Mu and Tn*9*, the only elements known to generate cointegrates during trans-

position, must account for the behavior of all transposable elements, and on a willingness to classify λ separately because its insertion is known to be conservative. The conservative nature of Mu insertion after infection juxtaposed with its replicative transposition after prophage induction, and consideration of the diversity of prokaryotic transposable elements in traits such as overall homology, size of target sequence duplications, and size of transposase proteins, indicate that insistence on a unified mechanism of insertion for all elements may not be justified.

The search for cointegrates between pBR322::Tn*5* plasmids and an F factor reported here was undertaken to determine whether it is necessary to invoke replicative models to explain the movement of Tn*5*.

## Tn*5* DOES NOT ENCODE A RESOLUTION FUNCTION

In principle the failure to find Tn*5*-mediated cointegrates between λ and the *E. coli* chromosome (BERG 1977) could have been ascribed to efficient resolution, as in the case of Tn*3*, or to loss of cointegrates if they are detrimental. Findings that several types of pBR322::Tn*5*-related plasmids with direct repeats of IS*50* or of Tn*5* are stable in *recA*$^-$ cells (HIRSCHEL & BERG 1982; SASAKAWA & BERG 1982; HIRSCHEL et al. 1982) suggested that Tn*5* elements do not undergo resolution comparable to that of Tn*3* elements. However, the finding that Tn*501* (Mer) encodes a resolvase which is expressed only after induction by mercuric ion (KITTS et al. 1982) emphasized the value of a more detailed search for a putative Tn*5*-encoded resolvase. Our test took advantage of two characteristics of transcription from the $P_L$ promoter of phage λ. (i) Transcription is repressed by the λ *c*I repressor, and is derepressed by shifting cells containing the *c*I857 temperature sensitive allele from 30°C to 41°C. (ii) Transcription initiated at $P_L$ in the presence of the λ *N* protein can continue unabated past most natural termination signals including those within Tn*5*. Consequently, insertion of $P_L$ into PBR322 can result in a plasmid useful for maximizing the expression of cloned genes (REED 1981; REMAUT et al. 1982). To search for a putative Tn*5* encoded resolvase (i) we transposed Tn*5* to a position near the $P_L$ promoter in plasmid p 8 of Reed (1981), (ii) generated a derivative in which the orientation of Tn*5* was reversed (Fig. 3), (iii) generated dimers of the pair of p 8::Tn*5* plasmids by homologous recombination in *recA*$^+$ bacteria, (iv) transformed them into a *recA*$^-$ strain carrying genes encoding *N* protein and the thermolabile *c*I857 λ phage repressor, and (v) turned on expression of *N*, and of the Tn*5* sequences downstream from $P_L$ in the plasmid by thermal inactivation of repressor. Fig. 4 shows that dimers of pλ8::Tn*5* plasmids are stable in *recA*$^-$ cells, and are not converted to monomers by induction of Tn*5* transcription. We interpret that Tn*5* does not encode a resolution function, and that consequently any cointegrates generated during transposition involving Tn*5* containing plasmids should be detectable.

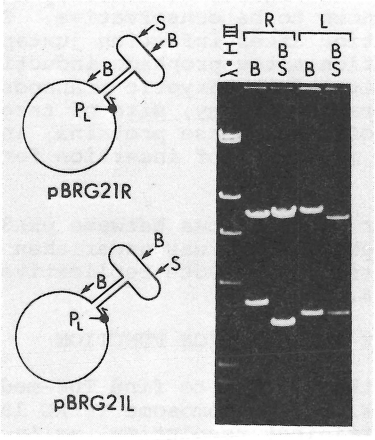


FIGURE 3. p 8::Tn*5* plasmids. Left: Maps of the pλ8::Tn*5* plasmids designated pBRG21R and pBRG21L, which differ only in the orientation of Tn*5* insertion. Right, BamHl and BamHl plus SmaI digestions which confirm the opposite orientations of Tn*5* in these two plasmids. The pλ8 vector used in these experiments is a pBR322 derivative containing an 1100 bp segment from phage λ which includes the leftward promoter and *N* gene of λ inserted into the BamHl site of pBR322 (REED 1981). pBRG21R was generated by transposition of Tn*5* from a chromosomal site to pλ8, selected by extraction of plasmid DNA and transformation of a recipient of $Kan^r$. pBRG21L resulted from a rare spontaneous in vivo inversion of Tn*5*.

## IS50 TRANSPOSITION DOES NOT GENERATE COINTEGRATES

A search for cointegrates between pBR322::Tn*5* and an F factor was undertaken to determine if it was necessary to invoke a replicative scheme for the movement of IS*50*-based elements. Electrophoresis of plasmid DNAs from *recA*$^-$ cells harboring pBR322::Tn*5* revealed homogeneous populations consisting of monomers or dimers of these plasmids (see HIRSCHEL & BERG 1982). This homogeneity made *recA*$^-$ strains ideal for tests of transposition mechanisms outlined in Fig. 5. If transposition is conservative, the movement of Tn*5* sequences from monomeric pBR322::Tn*5* plasmids to F should not be associated with cotransposition of pBR322 vector sequences. In contrast, transposition of Tn*5* and of pBR322 sequences from dimeric plasmids should often occur together.

Transposition to the F factor pOX38 was selected by crossing *recA*$^-$ cells containing pOX38 and pBR322::Tn*5* with an F$^-$ *recA*$^-$ recipient and selecting $Kan^r$ exconjujugants. The presence of vector sequences was scored by testing exconjugants for the $Amp^r$ marker of pBr322. When strains carrying monomeric pBR322::Tn*5* plasmids were used only about 1% of $Kan^r$ exconju-

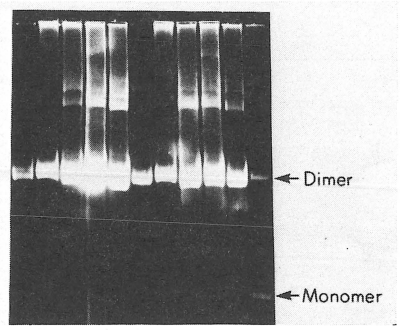


FIGURE 4. Derepressed transcription of Tn*5* does not lead to *recA*-independent recombination between directly repeated Tn*5* elements. Dimeric forms of pBRG21L and pBRG21R (Fig. 3) constructed by homologus recombination in vivo were introduced into a *recA*$^-$ derivative of bacterial strain N4830 (REED 1981) which carries a $\lambda$ prophage segment including *c*I857 and the *N* gene. Cultures were grown at 30°C (*c*I repressor active), shifted to 41°C (*c*I repressor inactive, N expressed), and plasmid DNA was extracted after 0, 1, 2, 3, and 4 hrs of growth at 41°C and electrophoresed in a 0.7% agarose gel (pBRG21R in lanes 1-5; pBRG21L in lanes 6-10). Neither growth overnight at 30°C of cells which had been heat shocked for 4 hours nor a similar heat shock treatment of cells carrying another $P_L$ promoter plasmid, pLC236 (REMAUT et al. 1981), into which we had also inserted Tn*5*, resulted in detectable conversion of dimeric to monomeric DNAs.

gants were Amp$^r$, whereas when dimeric plasmids were used about half of the Kan$^r$ exconjugants were Amp$^r$ (Table 1).

Electrophoresis of plasmid DNA extracted from individual Kan$^r$ Amp$^r$ exconjugant clones showed that it consisted of single molecular species somewhat larger than the pOX38 target. Digestion with BamHl, and enzyme which cleaves at single sites in pBR322 and in Tn*5*, was used to deduce the structures of representative pOX38-pBR322::Tn*5* chimaeras. The patterns of fragments obtained (see Fig. 6) indicated that each of the 59 independent chimaeras tested contained inserts of just one copy of pBR322 sequences bracketed either by one copy of IS*50* and one copy of Tn*5* (Fig. 5B,D), or by direct repeats of Tn*5* (Fig. 5D). We conclude that true cointegrates containing inserts of complete copies of a donor replicon plus duplicate copies of one or more IS*50* elements are rarely if ever formed as the result of IS*50* transposition.

## CIS ACTION OF Tn*5*'S TRANSPOSASE

Studies of complementation between Tn*5* and its derivative Tn*5*-410 (Trp$^+$) (Fig. 1) for transposition to $\lambda$ *red*$^-$ were undertaken in recombination deficient (*recA*$^-$) cells to better understand the topology of transposase action. Table 2 show that

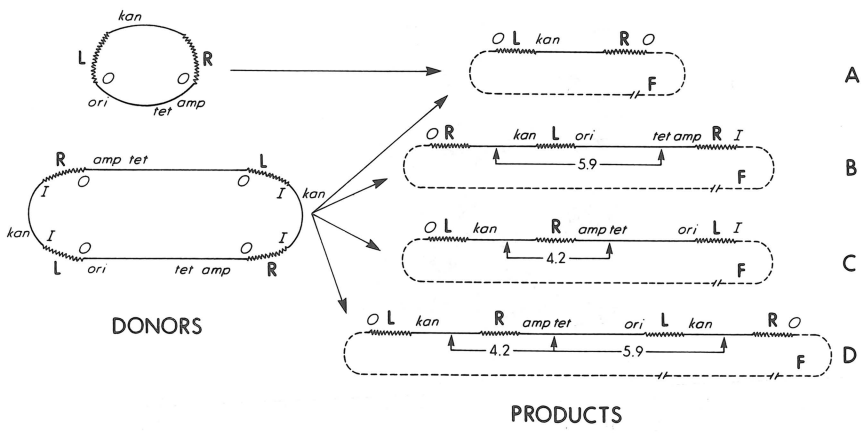


FIGURE 5. Predicted patterns of conservative transposition from monomeric and dimeric pBR322::Tn*5* plasmids to an F factor. The donor DNAs diagrammed at left are monomeric (top) and dimeric (bottom) pBRG11R plasmids. The structures of IS*50* mediated insertions into the F factor pOX38 and the sizes in kb of the diagnostic DNA fragments generated by BamH1 digestion (HIRSCHEL & BERG 1982) are indicated at right. Jagged lines, IS*50*; *O* and *I*, outside and inside ends of IS*50*, respectively (see Fig. 1; BERG et al 1982).

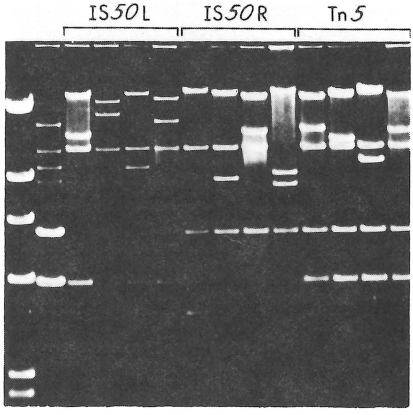


FIGURE 6. Restriction endonuclease analyses of representative pOX38-pBRG11R chimaeras selected as described in Table 1. The leftmost two lanes contain HindIII digested $\lambda^+$ and BamH1 digested pBRG11R DNA, respectively. The other lanes contain BamH1 digested pOX38-pBr322::Tn*5* chimaeric DNAs. IS*50*L, IS*50*R and Tn*5* refer to elements inferred to be at the junctions with pOX38 DNA using the rationale outlined in Figure 5.

Table 1. COTRANSPOSITION OF pBR322 WITH Tn*5*

| Plasmid Size: | Fraction Kan$^r$ with Amp$^r$ of pBR322 | |
|---|---|---|
| | Monomer | Dimer |
| Plasmid Designation | | |
| pBRG11R | .014 | .48 |
| pBRG11L | .005 | .53 |
| PTn*5*-DR1 | .010 | .45 |

Clones of *recA*$^-$ strain DB1986 carrying the indicated pBR322::Tn*5* plasmids were crossed with F$^-$ recipient DB1648 and Kan$^r$ and Str$^r$ exconjugants were selected and tested for Amp$^r$. Each entry in the table is based on the results of crosses with 12 or more separate donor clones. 50-200 exconjugants were tested from each cross involving a monomeric donor, and 15-20 were tested from each cross involving a dimeric donor. The structures of pBRG11L and pBRG11R, plasmids, which carry Tn*5* at the same site but in opposite orientations, are shown in Fig. 10, left. pTn*5*-DR1 contains direct instead of inverted repreats of its component IS*50* elements, and is derived from pBRG11L by BamH1 cleavage and ligation in vitro (HIRSCHEL & BERG 1982).

when Tn*5* and Tn*5*-410 are on different replicons, Tn*5*-wild type does not complement Tn*5*-410 for transposition. In contrast, when Tn*5* and Tn*5*-410 are physically linked as part of the same DNA molecule, either a multicopy pBR322 plasmid (pBRG28 in Fig. 7) or a single copy F' episome, complementation of Tn*5*-410 is efficient. This implies that transposase acts only on those IS*50* sequences present in the DNA molecule from which it was transcribed and translated.

## GENERALIZED INHIBITION OF Tn*5* TRANSPOSITION

Studies of bacteria carrying Kan$^s$ derivatives of a resident Tn*5* element had indicated that a Tn*5* element, once established in a cell, causes a partial inhibition of additional Tn*5* transposition. Preliminary tests using strains which seemed to contain deletions of part of a resident Tn*5* element were interpreted according to a model in which both IS*50*R and IS*50*L were necessary for this inhibition (BIEK & ROTH 1980). To test this interpretation and examine the inhibition phenomenon in greater detail we determined the frequency of transposition of Tn*5* from a λ::Tn*5* phage after infection of cells harboring pBR322::IS*50* plasmids. Table 3 shows that the presence of IS*50*R, the element which encodes transposase, lowers the frequency of transposition of Tn*5* about fifty-fold. In contrast, IS*50*L, which does not encode a functional transposase, does not inhibit Tn*5* transposition. To explore the idea that the trans-

Table 2. CIS ACTION OF Tn*5* TRANSPOSASE

| Location of Tn*5* | Transposition Frequency x $10^6$ | |
|---|---|---|
| | Tn*5* (Kan$^r$) | Tn*5*-410 (trp) |
| TRANS | | |
| F'*lac*::Tn*5*-410/chromosome::Tn*5*-WT | 6 | <.0001 |
| F'*lac*::Tn*5*-410/pBR322::Tn*5*(L) | 4 | <.0001 |
| F'*lac*::Tn*5*-410/pBR322::Tn*5*(R) | 8 | <.0001 |
| CIS | | |
| F'*lacP*::Tn*5*, lacY::Tn*5*-410 | 3 | 0.2 |
| mixed dimer pBR322::Tn*5*-410,Tn*5*(R) | 4 | 0.8 |
| mixed dimer pBR322::Tn*5*-410,Tn*5*(L) | 8 | 2.5 |

Transposition of Tn*5* or Tn*5*-410 to λ occurring in derivatives of *recA*$^-$ λ *rec*$^-$ $c^{ts}$ lysogen DB1873 was detected by thermal induction of the prophage and transduction of strain DB1891 to Kan$^r$ or Trp$^+$ phenotypes. The location of Tn*5* in the chromosome of the first strain was not determined. The F'*lac*204::Tn*5*-410 episome has been described (BERG et al. 1980b). pBR322::Tn*5*(L) and pBR322::Tn*5*(R) designate monomeric forms of plasmids pBRG11L and pBRG11R respectively (see Fig. 10). The mixed dimers were derived by recombination between the pBRG27 [a pBR322::Tn*5*-410 plasmid generated by replacement of Tn*5*-wild type in pBRG11L (see Fig. 10) with Tn*5*-410, and then homologous recombination between pBRG27 and pBRG11 plasmids, selected by stable coexistence and joint transformation of Trp$^+$ and Kan$^r$ traits. The structure of one hybrid plasmid, designated pBRG28L, is shown in Fig. 7.

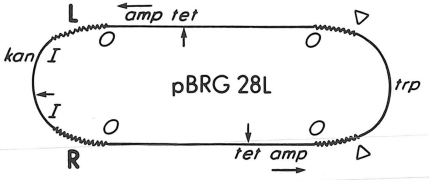


FIGURE 7. Structure of pBRG28L. This mixed dimer plasmid was constructed by homologous recombination in vivo between monomeric pBRG11L (Fig. 10) and pBRG27, a derivative of pBRG11L containing Tn*5*-410 (Fig. 1) in place of Tn*5* wild type.

posase gene product is responsible for inhibition we generated deletions of the internal end of IS*50*R in the plasmid shown in Fig. 8A using restriction endonucleases BglII and GclI (Fig. 8B), and tested the abilities of these deletion derivatives to inhibit Tn*5* transposition.

Table 3. INHIBITION OF Tn*5* TRANSPOSITION

| Plasmid | Characteristics | Transposition Frequency x $10^5$ |
|---|---|---|
| None | No IS*50* | 2.4 |
| pBR322 | No IS*50* | 1.65 |
| pBRG553 | IS*50*R normal transposase | 0.026 |
| pBRG556 | IS*50*L truncated transposase | 1.4 |
| pBRG557 | IS*50*R BclI/BamHI fusion normal transposase | 0.028 |
| pBRG554 | IS*50*R BglII/BamHl fusion elongated transposase | 0.74 |

Inhibition was monitored by comparisions of frequencies of transposition of Tn*5* from phage λ*b*221 *c*I857 *O*am8 *P*am80 *c*I857 *rex*::Tn*5*, which is defective in integration and replication, to the chromosome of *recA*⁻ strain DB1977 carrying the plasmids indicated. The position of IS*50*R in pBR322, and the design of the construction of the BclI/BamHl and BglII/BamHl fusions is outlined in Fig. 8. From the known sequence of IS*50* (AUERSWALD et al. 1980; see Fig. 8) and of pBR322 (SUTCLIFFE 1978), we infer that the BglII/BamHl fusion generates a full length transposase with an 80 amino acid carboxyterminal extension.

Because of the positions of the BglIIsite in IS*50* (Fig. 8C) and the compatible BamHl site in pBR322, the BglII/BamHl fusion should encode an elongated protein which contains a complete transposase fused to 80 amino acids encoded by sequences in the $tet^r$ region of pBR322 (AUERSWALD et al. 1980; SUTCLIFFE 1979). A comparable fusion using the BclI site just 5 bp downstream from the BglII site results in a normal length transposase protein because the TGA stop codon at the end of the transposase gene is preserved (Fig. 8C). The BglII/BamHl fusion plasmid (pBRG554) inhibits Tn*5* transposition very weakly, whereas the Bcl/BamHl fusion plasmid (pBRG557) exerts normal inhibition of Tn*5* transposition (Table 3). Thus the product of the transposase gene, but not the sequence of inside end of IS*50* is essential for IS*50*-mediated inhibition of Tn*5* transposition. Future experiments should elucidate how transposase can both mediate and inhibit transposition, and how the form of the trans-

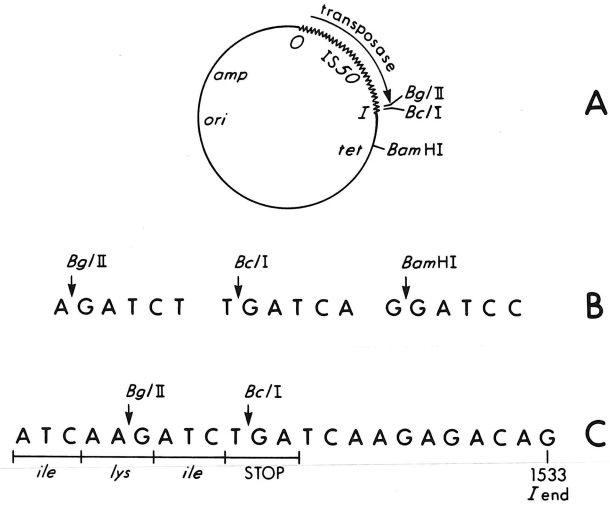


FIGURE 8. Strategy for deletions of the inside *I* terminus of IS*50*. A. Diagram of the pBR322::IS*50* plasmid (BERG et al. 1982) containing IS*50* at the beginning of the $tet^r$ gene chosen for these deletion experiments. B. The sequences (on one strand) of the recognition sites of three restriction endonucleases which generate 4 bp extensions of GATC. C. The sequence of the inside end of IS*50*, including the last codons of the transposase gene (AUERSWALD et al. 1980; BERG et al. 1982).

posase gene product which promotes IS*50* and Tn*5* movement only in *cis* (Table 2) differs from that which inhibits the transposition of Tn*5* in *trans* (Table 3).

## TRANSCRIPTION DECREASES IS*50* TRANSPOSABILITY

We examined the structure of chimaeric molecules generated by transposition from pBR322::Tn*5*-related plasmids to phage $\lambda red^-$ in order to identify factors determining the participation of IS*50*R and IS*50*L in transposition. The types of plasmids used are indicated in Fig. 9; dimeric forms were used in *recA*$^-$ cells because of the conservative pattern of IS*50*-mediated transposition events (Fig. 5; Table 1). Phage $\lambda red^-$ was used as the target for transposition instead of pOX38 because preliminary experiments (HIRSCHEL & BERG 1982; SASAKAWA & BERG 1982) had shown that its relatively small genome size permitted the efficient packaging of pBR322::Tn*5* sequences bracketed by direct repeats of IS*50* (11.5kb), but not the larger (16 kb) element containing terminal repeats of Tn*5*. Digestion of the resulting λ-pBR322::Tn*5* chimaeric DNAs with BamHI generated fragments whose sizes indicated whether transposition had been

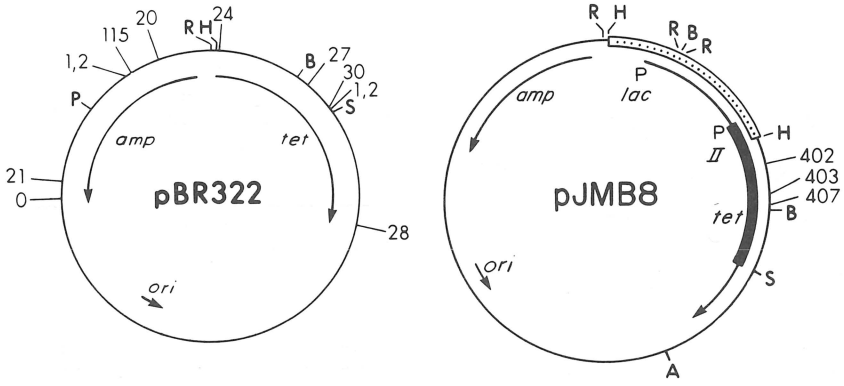


FIGURE 9. Plasmids used in analyses of transcriptional control of the participation of IS*50*R and IS*50*L in transposition. pJMB8 is a derivative of pBR322 containing in insert of a 1000 bp segment including *lac* and M13 gene II promoters in the HindIII site. Restriction endonuclease cleavage sites are indicated: R, EcoRI; HIII, HindIII; B, BamHl; Sa, SalI, Sm; A, AvaI. The sites of Tn*5* insertion are indicated by the allele numbers (see Table 5-8). The intense transcription of the first part of the *tet*$^{r}$ locus is indicated by the thickened arrow in pJMB8. Transposition of Tn*5* from the *E. coli* chromosome to pBR322 and to pJMB8 was selected based on the very high levels of neomycin resistance (>250 µg/ml) that Tn*5* confers when it is in a multicopy plasmid. The sites and orientation of Tn*5* insertion were mapped by restriction endonuclease digestion as described previously (HIRSCHEL & BERG 1982).

mediated by a pair of IS*50*R elements, or by a pair of IS*50*L elements (Fig. 10).

In our first experiments, among 56 independent cointegrates generated by transposition from the pBR322::Tn*5* plasmid pBRGllR, 27 were formed using pairs of IS*50*R elements, and 29 were formed using pairs of IS*50*L elements. In contrast, among 71 independent cointegrates formed by transposition from pBRGllL, a plasmid identical to pBRGllR above except for the orientation of the Tn*5* element relative to pBR322 sequences (see Fig. 10), 70 were formed using pairs of IS*50*R elements, and only 1 was formed using a pair of IS*50*L elements. The difference between the 27:29 (pBRGllR) and the 70:1 (pBRGllL) ratios of IS*50*R:IS*50*L usage indicated that the orientation of Tn*5* at a single site in a donor plasmid affects the relative transposability of its component IS*50*L and IS*50*R elements. Representative digests are shown in Fig. 11.

To better assess the site-specificity of IS element usage the additional dimeric plasmids indicated in Fig. 9A were gen-

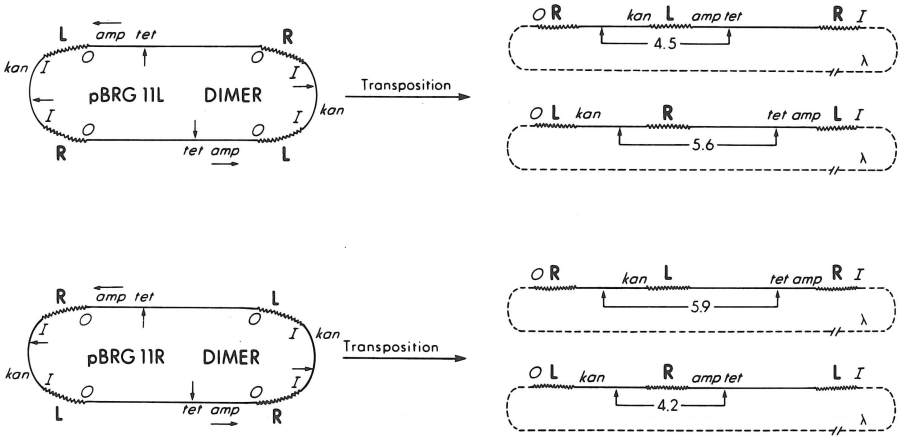


FIGURE 10. Predicted structures of λ-pBR322::Tn*5* chimaeras resulting from transposition from dimeric pBRG11L and pBRG11R. The sizes in kb of the distinctive BamHl fragments which distinguish cointegrates generated by pairs of IS*50*R elements from those generated by pairs of IS*50*L elements are indicated. Plasmids pBRG11L and pBRG11R differ only in the orientation of Tn*5* with regard to pBR322 (HIRSCHEL & BERG 1982).

erated and used as donors in transposition. The results (Table 4) showed that IS*50*R mediated the majority of transposition events when Tn*5* was in the *tet*$^r$ locus in either orientation, and when Tn*5* was in the *amp*$^r$ locus oriented such that IS*50*L was closest to the *amp*$^r$ promoter. In contrast, when Tn*5* was in the opposite orientation in the *amp*$^r$ locus transpositions mediated by IS*50*R and IS*50*L occurred at about the same frequency.

Because the *amp*$^r$ promoters are several fold stronger than the *tet*$^r$ promoter of pBR322 (STUBER & BUJARD 1981), these results suggested an effect of transcription of IS*50* transposition. Transcription might inhibit the use of IS*50* in transposition directly, for example, by dislodging bound transposase or by altering DNA structure. Alternatively, transposase made from transcripts initiated at the promoter within IS*50*R might act preferentially on IS*50*R, whereas transposase made from longer transcripts initiated at vector promoters might not be as constrained spatially and thus might act on IS*50*L as well as on IS*50*R. Only the first explanation predicts that placement of Tn*5* near very strong promoters could lead to the preferential use of IS*50* in transposition.

These alternatives were tested using plasmid pJMB8, a pBR322 derivative whose *tet*$^r$ locus is transcribed from very strong promoters of M13 gene II and the *E. coli lac* operon

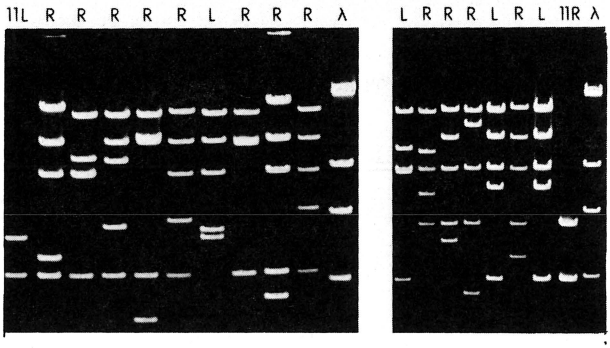


FIGURE 11. Agarose gel electrophoresis of BamH1 digests of λ Amp$^r$ Tet$^r$ Kan$^r$ chimaeras resulting from transposition from dimeric pBRG11L and pBRG11R plasmids to phage λ. R and L markers above th lanes indicate IS*50*R and IS*50*L elements which participated in transposition, inferred from the rationale outlined in Fig. 10.

(J. BUZAN, pers. comm.). Using dimeric forms of three pJMB8*tet*::Tn*5* plasmids (Fig. 9B) we found that IS*50*R participated only about 10-20% as efficiently as IS*50*L in transposition. Reversal of the orientation of Tn*5* resulted in preferential use of IS*50*R similar to that seen previously with pBR322::Tn*5* (Table 5). We conclude that transcription directly inhibits transposition.

ORIENTATION OF Tn*5* INSERTION

The distribution of orientations of new insertions of Tn*5* in certain regions of pBR322 was surprisingly nonrandom. IS*50*R was closest to the promoter in 19 of 23 independent Tn*5* insertions in *amp*$^r$. In the *tet*$^r$ locus, in constrast, insertions in both orientations were equally frequent (Table 6). Because *amp*$^r$ is transcribed more strongly than *tet*$^r$ in pBR322 (STUBER & BUJARD 1981), this result suggested models in which transcription of the target molecule might also play a role in controlling transposition. In support of this interpretation, among insertions in the strongly transcribed *tet*$^r$ locus of pJMB8, IS*50*R also tended to be nearest the promoter (Table 6).

## DISCUSSION

New insights into the mechanism and control of Tn*5* transposition have been provided by our experiments.

CONSERVATIVE TRANSPOSITION

Because Tn*5* does not encode an active resolution function cointegrates if formed during IS*50* transposition from pBR322::Tn*5* plasmids should be detectable. Cointegrates are not formed

Table 4: IS*50* USAGE IN TRANSPOSITION FROM pBR322::Tn*5*

| | | Element Closest to pBR322 Promoter | | | | |
|---|---|---|---|---|---|---|
| | | IS*50*R | | | IS*50*L | |
| Site | Plasmid | IS*50*R Used | IS*50*L Used | Plasmid | IS*50*R Used | IS*50*L Used |
| *tet*24 | pBRG60R | 35 | 0 | -- | -- | -- |
| *tet*27 | --- | -- | -- | pBRG63L | 35 | 1 |
| *tet*30 | pBRG65R | 25 | 11 | -- | -- | -- |
| *tet*1 | pBRG14R | 48 | 8 | pBRG14L | 37 | 6 |
| *tet*2 | pBRG15R | 33 | 3 | pBRG15L | 31 | 5 |
| *tet*28 | pBRG64R | 30 | 2 | -- | -- | -- |
| TOTAL | | 171 | 24 | | 103 | 12 |
| *amp*20 | pBRG51R | 13 | 18 | -- | -- | -- |
| *amp*115 | pBRG57R | 17 | 15 | -- | -- | -- |
| *amp*1 | pBRG12R | 17 | 17 | pBRG12L | 33 | 3 |
| *amp*2 | pBRG13R | 24 | 9 | pBRG13L | 35 | 1 |
| *amp*21 | pBRG52R | 16 | 19 | -- | -- | -- |
| *amp*0 | pBRG11R | 27 | 29 | pBRG11L | 70 | 1 |
| TOTAL | | 114 | 107 | | 138 | 5 |

Transpositions of pBR322::Tn*5* sequences to λ *red*$^-$ in *recA*$^-$ *E. coli* were selected, and the pairs of IS*50* elements which mediated the transposition were inferred by BamH1 digestion of λ-pBR322::Tn*5* chimaeric phage DNAs as in Fig. 10,11. The sites of Tn*5* insertion into pBR322 are shown in Fig. 9. Tn*5* was inverted without changing its site of insertion in several plasmids by ligation after digestion with a restriction endonuclease which cleaves IS*50* exterior to the sequence difference between IS*50*R and IS*50*L. HpaI was used for plasmids pBRG12-pBRG15 (this work) and HindIII was used for pBRG11 (HIRSCHEL & BERG 1982).

Table 5. TRANSCRIPTION FROM STRONG PROMOTERS INHIBITS TRANSPOSITION DIRECTLY

| | Allele | Plasmid | IS*50*R used | IS*50*L used |
|---|---|---|---|---|
| A. IS*50*R closest to promoters | | | | |
| | *tet*402 | pBRG302R | 3 | 33 |
| | *tet*403 | pBRG303R | 5 | 31 |
| | *tet*407 | pBRG307R | 5 | 36 |
| | | TOTAL | 13 | 100 |
| B. IS*50*L closest to promoters | | | | |
| | *tet*403 | pBRG303L | 35 | 1 |

λpJMB8::Tn*5* chimaeras were selected and analyzed as described in Table 4. Plasmids pBRG303R (part A) and pBRG303L (part B) are identical except for the orientation of Tn*5*.

during transposition from monomeric pBR322::Tn*5* plasmids. Although Tn*5* and pBR322 vector sequences are frequently transposed together from dimeric plasmids, these transposition products contain just a single copy of pBR322 sequences bracketed by an IS*50* and a Tn*5* element or by a pair of Tn*5* elements, rather than complete copies of the entire dimeric pBR322::Tn*5* plasmid. Thus IS*50*-mediated transposition seems to always involve just a segment of the donor molecule.

The failure to detect conintegrates rules out simple bidirectional replicative models (SHAPIRO 1979; ARTHUR & SHERRATT 1979) as explanations of IS*50* transposition. The data is compatible with unidirectional rolling circle replicative models if: (i) The pattern of joining of strands at the termination of IS*50*-mediated transposition is always of the type which fails to generate cointegrates. (ii) IS*50* segments which have replicated once during a transposition event are marked to preclude their being replicated again in the same event. However, given the precedents of conservative insertion of λ (NASH 1981) and also of phage Mu (LIEBHART et al. 1982), one of the elements for which a rolling circle replicative model had been devised (BUKHARI 1981), a model in which IS*50*-mediated transposition is conservative (Fig. 2) is, for us, the simplest interpretation. We envision that transposition occurs by breakage of donor and target replicons and the insertion of a segment into the target replicon without replication. The remainder of the donor does not recircularize and is lost, and DNA synthesis is limited to that necessary to repair the single-stranded gaps (see Fig. 2; BERG 1977).

Table 6. ORIENTATION OF Tn*5* INSERTIONS

| Tn*5* Location | Promoter | Element Closest to Promoter | |
|---|---|---|---|
| | | IS*50*R | IS*50*L |
| pBR322 *amp*$^r$ | strong | 19 | 4 |
| pBR322 *tet*$^r$ | weak | 22 | 22 |
| pJMB8 *tet*$^r$ | strong | 19 | 3 |

Transposition of Tn*5* from site *lacZ*124 (BERG et al. 1980b) in the chromosome of F$^-$ *recA*$^-$ strain DB1572 were selected by growth in ampicillin and tetracycline-free broth containing very high levels (250 g/ml) of neomycin. The locations of insertions which caused Amp$^S$ and Tet$^S$ phenotypes were mapped relative to HindIII, BamHI and/or SmaI sites in the pBR322 and pJMB8 plasmids (see Fig. 9), and then the orientations of the Tn*5* elements were determined from single and double digestions as in Fig. 3.

Whereas Tn*5* may always transpose by a conservative mechanism, other elements such as Tn*9* which often generates cointegrates (GALAS & CHANDLER 1982; BIEL & BERG in preparation) may be able to undergo both replicative and conservative transposition. Cointegrates would be the consequence of replicative transposition, and simple transposition of Tn*9* without cointegrate formation might be the consequence of a double-strand break and consequent loss of the donor. The relative frequencies of the two types of transposition products might reflect the kinetics of the onset of replication relative to that of converting a single strand nick to a double strand break (BIEL & BERG in preparation).

In principle destruction of the donor molecule during conservative transposition might result in its disappearance from clones in which transposition has taken place. However, a consequence of the multiple copies of most replicons in the average bacterial cell is that transposition should rarely if ever lead to genetically identifiable loss of a replicon. Destruction of one copy would permit a sibling molecule which had not participated in transposition to replicate precociously, thereby establishing a lineage with the element at both old and new locations.

Part of the intuitive appeal of replicative transposition as exhibited by Tn*3* stems from the notion that successful transposable elements are selfish DNAs which transpose by mechanisms which increase their copy number relative to other genomic sequences (DOOLITTLE & SAPIENZA 1980). However, conservative transposition also leads to a disproportionate increase in an element's frequency in the DNA population, and hence is also likely to confer an important benefit to elements which utilize

it.

## CIS ACTING TRANSPOSASE

The inability of the Tn*5* encoded transposase to complement the defective Tn*5*-410 element documented here corrects the interpretation of MEYER et al. (1979) that Tn*5*'s transposase acts in *trans*. Since these workers had used recombination proficient *E. coli* cells (in contrast to our use of *recA*$^-$ *E. coli* and *red*$^-$ λ phage; see Table 1 legend), their results can be ascribed to recombination between homologous sequences in Tn*5* and Tn*5*-410 (see BERG 1980), followed by *cis* complementation equivalent to that reported in Table 2. Limiting the action of transposase to sequences *cis* to the gene encoding it might occur if, during its synthesis, transposase wrapped around the Tn*5* containing DNA molecule in the form of a donut (Fig. 12). This complex might be free to migrate to IS*50* elements elsewhere on the same DNA molecule, but not to other replicons.

Ideas of *cis* acting transposases, especially ones which act preferentially on the IS element which encodes them, are intriguing in the context of the selfish DNA concept. Since multiple copies of an element accumulate in cell lineages, and since its transposition may often be repressed, selection would favor IS elements which when transiently or accidentally derepressed made transposase proteins which diffused very little and acted preferentially on the DNA segments from which they were synthesized.

## NONRANDOM ORIENTATION OF Tn*5* INSERTION

The findings that IS*50*R is usually closest to the promoters in the activity transcribed *amp*$^r$ locus of pBR322 and *tet*$^r$ of pJMB8 can be explained in the context of the donut model of transposase action. We propose that the newly synthesized transposase complex binds initially to IS*50*R and to a potential target, and that target sequences become activated for insertion by transcription, possibly because of transient denaturation of transcribed DNA. Completion of transposition may await activation of the chosen target by events such as transcription, during which time migration of the Tn*5* segment through the transposase complex permits contact with IS*50*L, and hence the randomization of Tn*5* orientation such as that seen among insertions in the transcriptionally quiescent *tet*$^r$ gene of pBR322.

## GENERALIZED INHIBITION OF Tn*5* TRANSPOSITION

Our experiments indicate that the product of the transposase gene, necessary for movement of IS*50* and Tn*5* is also necessary for inhibition of Tn*5* movement (Table 3). Whereas the transposition activity is evident only in *cis*, inhibition operates in *trans*.

How might the product of a single gene exhibit such contrasting activities? Translation of the transposase message is initiated in the same reading frame at two sites, and generates a pair of proteins 461 and 421 amino acids long (see JOHNSON &

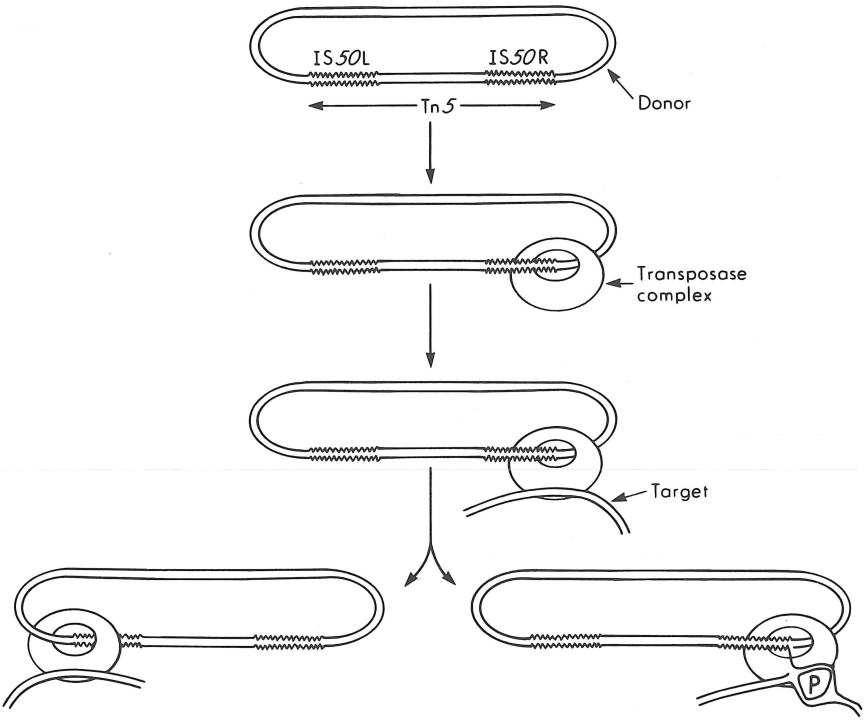


FIGURE 12. The donut model for transposase action. Transposase protein is postulated to bind to and wrap around the DNA molecule containing IS*50*R, during or following coupled transcription and translation. It is able to move along the IS*50*R containing molecule, and thus act on IS*50*L. It can not act on IS*50* elements present on separate DNA molecules (Table 3). The site on transposase used for binding to target DNA, which is not specific for any particular DNA sequence, is postulated to lie on an outer surface of the protein. A possible role for RNA polymerase (designated P) or transcription in the target DNA suggested by the data in Table 6, is also presented.

REZNIKOFF 1981). The larger protein is essential for transposition (ROTHSTEIN et al. 1980), and perhaps it is the smaller which mediates inhibition. Alternatively inhibition could be due to degraded or processed products of full length transposase, or simlpy to those transposase polypeptides which do not properly fold into the functional transposase complex during synthesis. Inhibition might operate by the tight binding of IS*50*, thereby blocking access of the functional transposase complex to these sites. Alternatively, inhibition might act at the transcriptional level, since the sequence of a segment of the transposase gene promoter resembles a region near IS*50*'s

ends (JOHNSON & REZNIKOFF 1981).

## CONTROL OF IS*50*R AND IS*50*L MEDIATED TRANSPOSITION EVENTS

Use of pJMB8 plasmids carrying Tn*5* downstream from the active *lac* operon and M13 gene II promoters as transposition donors established that transcription impinging on IS*50*R inhibits its use in transposition, and by default permits most transpositional events to be mediated by IS*50*L (Table 5). It is possible that transcriptional inhibition of transposition might be solely responsible for the patterns of IS*50*R and IS*50*L usage from all pBR322::Tn*5*-related plasmids (see Table 4). In this framework the preferential use of IS*50*R when Tn*5* is inserted in relatively quiescent regions of vector molecules would be ascribed to decreased activity of the inside end of IS*50*L caused by transcription from the $kan^r$ promoter. The equal frequency of IS*50*L and IS*50*R usage from pBR322*amp*::Tn*5* plasmids in which IS*50*R is closest to the promoter would then reflect the balanced inhibition caused by transcription from the $amp^r$ promoter across the outside end of IS*50*R, and from the $kan^r$ promoter across the inside end of IS*50*L.

Transcription at the end of an IS*50* element might inhibit its use in transposition in one of two ways: By creation of transiently single-stranded regions which are poor substrates for transposase, or by direct interactions between RNA polymerase and transposase, resulting in inhibition of transposase, or its displacement to another recognition site.

An alternative view ascribes part of the preference for usage of IS*50*R to a tendency for the Tn*5* encoded transposase to act preferentially on IS*50*R as implied by the donut model in Fig. 12.

## CONCLUSIONS

The experiments presented here have provided us with new insights into the mechanism and control of transposition. Because the transposition of Tn*5* between replicons does not result in the formation of true cointegrates between donor and target molecules, Tn*5* transposition appears to be a conservative process, not replicative as had often been assumed.

Complementation experiments pointed to extraordinary properties of the product of Tn*5*'s transposase gene. The product is necessary for transposition, and in this capacity appears to operate exclusively on the DNA molecule containing the gene encoding it (*cis*). A product of the transposase gene is also responsible for inhibiting Tn*5* transposition, and inhibition operates on any DNA molecule in the cell (trans). We propose that *cis* action reflects topological interlocking of transposase and DNA - a transposase complex shaped like a donut through which donor DNA is threaded. Migration of the DNA with respect to the protein enveloping it permits transposase to bind recognition sites on elements such as IS*50*L and Tn*5*-410 as well as on IS*50*R. Inhibition may demand a less ordered

structure than transposition, and could be mediated by an alternative or shortened form of transposase which binds IS*50* DNA tightly but lacks both the flexibility necessary for migration and the complete set of activities necessary for transposition.

The participation of individual IS elements in transposition is regulated in additional ways. Because IS*50* elements downstream from very active promoters transpose less frequently than those in transcriptionally quiescent regions, we conclude that transcription impinging on the end of an IS*50* element inhibits its transposition directly. Consequently, the preferential use of IS*50*R in transposition from plasmids containing Tn*5* in transcriptionally quiescent regions could reflect (i) a tendency for transposase to act on IS*50*R which encodes it, in preference to IS*50*L which does not, or (ii) inhibition of IS*50*L transposition by transcription from the $kan^r$ promoter across the inside end of IS*50*L.

The preferential insertion of Tn*5* in one orientation in transcriptionally active regions such that IS*50*R is closest to the promoter suggests that transcription of the target may activate it for insertion.

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