26. N. M. Maraldi, G. Barbanti-Brodano, M. Portolani, M. LaPlaca, *J. Gen. Virol.* **27**, 71 (1975).
27. B. Wasylyk, C. Wasylyk, P. Augereau, P. Chambon, *Cell* **32**, 503 (1983).
28. R. M. Lawn, E. F. Fritsch, R. C. Parker, G. Blake, T. Maniatis, *ibid.* **15**, 1157 (1978).
29. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
30. J. Messing and J. Vieira, *Gene* **19**, 269 (1982).
31. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); M. D. Biggin, T. J. Gibson, G. F. Hong, *ibid.* **80**, 3963 (1983).
32. M. Fried, M. Griffiths, B. Davies, G. Bjursell, G. La Mantia, L. Lania, *ibid.*, p. 2117.
33. M. Katinka, M. Yaniv, M. Vasseur, D. Blangy, *Cell* **20**, 393 (1980); F. K. Fujimura, P. L. Deininger, T. Friedmann, E. Linney, *ibid.* **23**, 809 (1981); M. Katinka, M. Vasseur, N. Montreau, M. Yaniv, D. Blangy, *Nature (London)* **290**, 720 (1981); K. Sekikawa and A. J. Levine, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1100 (1981); F. K. Fujimura and E. Linney, *ibid.* **79**, 1479 (1982).
34. S. Watanabe and K. Yoshiike, *J. Virol.* **42**, 978 (1982).
35. _____, A. Nozawa, Y. Yuasa, S. Uchida, *ibid.* **32**, 934 (1979).
36. R. Grosschedl and M. L. Birnstiel, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1432 (1980).
37. P. Mellon, V. Parker, Y. Gluzman, T. Maniatis, *Cell* **27**, 279 (1981); R. K. Humphries *et al.*, *ibid.* **30**, 173 (1982).
38. G. I. Bell, M. J. Selby, W. J. Rutter, *Nature (London)* **295**, 31 (1982); P. S. Rotwein *et al.*, *N. Engl. J. Med.* **308**, 65 (1983).
39. N. J. Proudfoot, A. Gil, T. Maniatis, *Cell* **31**, 553 (1982).
40. D. J. Capon, E. Y. Chen, A. D. Levinson, P. H. Seeburg, D. V. Goeddel, *Nature (London)* **302**, 33 (1983).
41. C. Queen, S. T. Lord, T. F. McCutchan, M. F. Singer, *Mol. Cell. Biol.* **1**, 1061 (1981).
42. S. E. Conrad and M. R. Botchan, *ibid.* **2**, 949 (1982).
43. J. Saffer and M. Singer, in press.
44. S. Subramani and J. Saffer, personal communication.
45. J. van der Noordaa, personal communication.
46. P. Gruss and G. Khoury, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 133 (1981).
47. We thank Y. Barra, J. Brady, R. Brent, D. Cosman, P. Howley, G. Jay, M. Kessel, L. Laimins, M. Martin, R. Muschel, R. Pozzatti, J. Savarese, and M. Singer for helpful discussions and editing; A. B. Rabson and R. Koller for gifts of AGMK and human DNA's; J. Duvall, M. Chang, M. Priest, and D. Hawkins for technical assistance. N.R. was supported by a Damon Runyon–Walter Winchell Cancer Fund fellowship.

Insertion Sequence Duplication in Transpositional Recombination

Ted A. Weinert, Nancy A. Schaus, Nigel D. F. Grindley

Summary. Insertion sequences (IS) are discrete segments of DNA that can transpose from one genomic site to another and promote genetic rearrangements. A question that is central to understanding the mechanism of transpositional recombination is whether genetic rearrangements are accompanied by duplication of the IS that promotes them. Analysis of adjacent deletions mediated by IS*903* provides the strongest evidence to date than any IS-mediated transpositional recombination can occur by an efficient replicative mechanism.

The genomes of a wide variety of eukaryotic and prokaryotic organisms contain segments of DNA that can move from one location to another and can mediate other genetic rearrangements. Indeed, these transposable elements (transposons) provide the molecular basis for the genetic instability that has puzzled and intrigued geneticists for several decades (*1*).

Many different transposons have been identified in Gram-negative bacteria and extensively characterized. Most of them fall into one of two groups (*2*): the rather homogeneous Tn*3* family of transposons, and the more heterogeneous collection of insertion sequences (IS) (together with the composite transposons that contain a segment of DNA flanked by two copies of an IS). Attempts to understand the mechanisms of transpositional recombination have focused on genetic and structural characterization of the transposons themselves and of the products formed during recombination. One of the primary questions has been whether a transposon is duplicated during transpositional recombination.

In the case of Tn*3* and related transposons there is a strong evidence that transposition is a replicative process (*3*). During the first stage of transposition, donor and target replicons are fused to form a cointegrate. This intermediate contains two copies of Tn*3*, one at each junction between donor and target DNA sequences (*4*). From this cointegrate, simple insertions can then be generated by a site-specific recombination between the two transposon copies. For the second group of transposons, the IS elements, the evidence for replicative transposition is much weaker. Although IS elements do promote the fusion of replicons, this occurs only infrequently (at about 1 percent of the frequency of simple insertions) (*5–7*), and once formed the resultant cointegrates are generally stable in a RecA$^-$ host strain. Simple insertions of an IS are therefore thought to be generated not by breakdown of a cointegrate intermediate but rather by a one-step process that results directly in the integration of a single IS copy at a target site. From the strong dependence of cointegrate formation on *recA* activity that is found with the IS*50* composite transposon, Tn*5* (*7*), it has been argued that the cointegrates observed may result from simple insertions from dimers of the donor replicon rather than from true replicon fusion between the target and a monomer of the donor (*8*). Apart from cointegrate formation, the only other overtly replicative transpositional process is "inversion-insertion" (also called "duplicative inversion") (*2*): the inversion of a DNA segment adjacent to a transposon, coupled to an insertion of the element in the opposing orientation at the other end of the inverted DNA segment (see Fig. 5B). However, only three examples of such IS-mediated inversion-insertions have been documented (two with IS*1* and one with IS*10*) and all were detected in RecA$^+$ hosts (*9, 10*). Thus the possibility remains that these rearrangements took place in two unlinked steps: an intermolecular insertion of the IS in inverted orientation, followed by *recA*-mediated recombination between the two IS copies to invert the intervening DNA segment.

With evidence for the apparently replicative IS transpositional processes of replicon fusion and inversion-insertion in some doubt, it is not surprising that evidence for a replicative process in the formation of simple insertions or a fourth IS-promoted genetic rearrangement, adjacent deletions, is essentially nonexis-

T. A. Weinert is a graduate student, N. A. Schaus was a postdoctoral fellow, and N. D. F. Grindley is an associate professor in the Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510. Nancy Schaus is currently a senior biologist in the Molecular Genetics Department, Lilly Research Laboratories, Indianapolis, Indiana 46285.

tent. Interestingly, bacteriophage Mu, representative of a third group of bacterial transposons (*2*), provides the first direct evidence that simple insertions may occur by a conservative, nonreplicative mechanism (*11*). Yet it is clear in the case of Mu that the major transpositional pathway is a replicative process since it is only by transposition that the Mu genome is replicated during the phage lytic cycle (*12*).

Analysis of IS*903*-Mediated Intramolecular Events

In an effort to gain more insight into the mechanism of IS transpositional recombination we have begun a systematic study of intramolecular rearrangements mediated by IS*903* in a host strain that is deficient in homologous recombination.

IS*903* is the component of the composite transposon Tn*903* that provides the functions and sites for transpositional recombination (*13*). The IS is 1057 base pairs (bp) in length, has a long open reading frame (307 codons) that encodes a transposase, and has perfect terminal inverted repeats of 18 bp (*13, 14*) (Fig. 1). Transposition of IS*903* or of Tn*903* results in duplication of a 9-bp target sequence with one copy at each end of the integrated element (*15*). Tn*903* transposes at a low frequency as a result of inefficient expression of the transposase gene. To increase the frequency of transpositional recombination and to ensure that expression of the transposase was not influenced by the presence of the IS*903* end adjacent to the start of the gene, we have constructed a derivative in which the transposase gene is transcribed from the lacUV5 promoter (Fig. 1A). When incorporated into a complete Tn*903*, this promoter fusion increased transposition by a factor of about 100 (*16*).

So that we can select for IS-mediated intramolecular rearrangements we have cloned a conditionally lethal gene close to IS*903* derivatives on a multicopy plasmid. The gene we have used is *galK*; expression of *galK* in a *galE*$^-$*T*$^-$ strain of *Escherichia coli* is lethal in the presence of galactose because toxic levels of galactose-1-phosphate accumulate. The plasmids pTW75 and pTW76 were constructed (see Fig. 2) and were introduced into the host strain NG135 (K12 *galΔS165 recA56 strA*) by transformation. Individual transformants were grown in Luria broth overnight to allow formation and segregation of rearrangements of the plasmids. Cells (10^7) from each overnight culture were plated on LB agar plates supplemented with 1 percent galactose (to select for events that inactivate *galK*) and carbenicillin (50 μg/ml) (to ensure retention of the plasmid). About 100 times as many survivors were obtained from cultures of cells harboring pTW75, the plasmid with an active IS*903*, as from those with pTW76, which contains a deleted transposase gene (Fig. 2). More than 95 percent of the galactose-resistant (Gal^R) cells that initially contained pTW75 simultaneously became sensitive to kanamycin. This suggested that a single deletion event was responsible for loss of both kanamycin resistance (Km^R) and *galK* expression. Extraction of plasmid DNA and analysis with restriction endonucleases showed that the pTW75 plasmids suffered deletions that spanned the region between the IS*903* and *galK* (Fig. 3). However, as expected from results obtained with other IS elements (*17*), the IS*903* itself was retained; a Taq I site 18 bp inside the deletion-proximal end was retained in every event analyzed. DNA sequence analysis confirmed that the

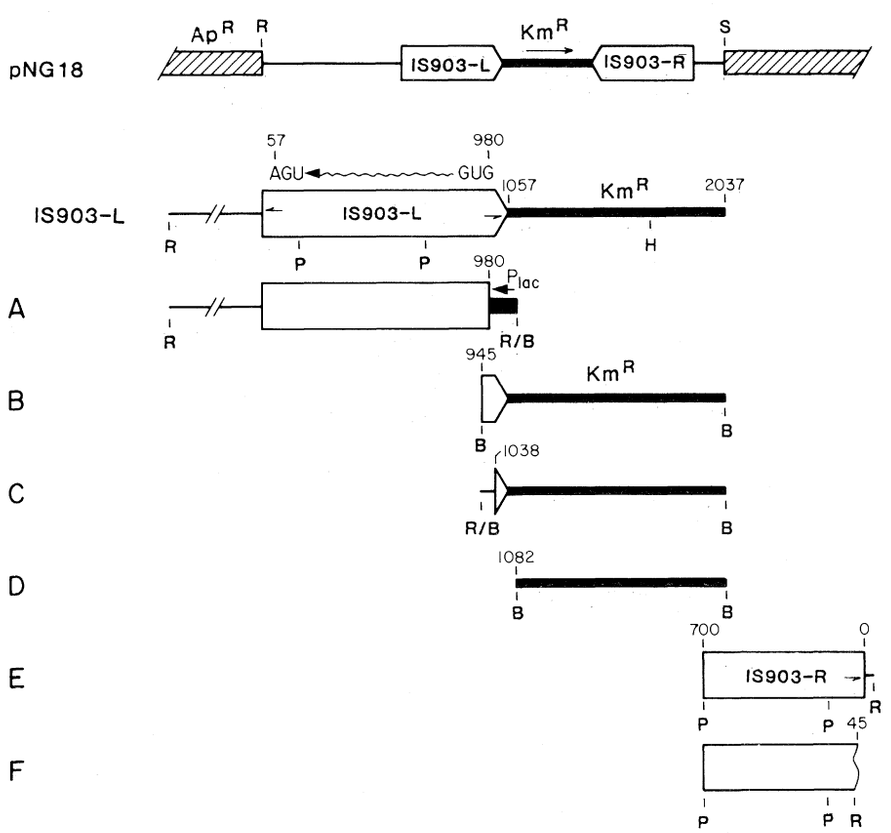
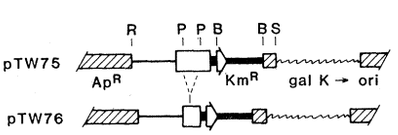


Fig. 1. Tn*903* and modules for construction of IS*903* derivatives. Restriction endonuclease cleavage sites are: B, Bam HI; H, Hind III; P, Pvu II; R, Eco RI; S, Sal I. The plasmid pNG18, a derivative of pBR322 that carries Tn*903*, has been described (*13*); pBR322 sequences are shown as shaded blocks. IS*903*-L and IS*903*-R are defined relative to the direction of transcription of the kanamycin resistance gene (Km^R) and to the Eco RI and Sal I sites. IS*903* contains a single long coding frame that starts with a GUG at position 980 and ends with the UGA at position 59; the translation product of this reading frame is absolutely required for transpositional recombination. The short half-arrows at each end of IS*903* indicate the 18-bp terminal inverted repeats. (A) The right end of IS*903*-L has been removed and replaced with a 100-bp Eco RI–Pvu II fragment from pGL101 (*33*) that carries the lacUV5 promoter. The fusion abuts the flush end of the Pvu II site to position 980 of IS*903* (the translational start site). Module A has either an Eco RI or a Bam HI site at the promoter-proximal end. (B to D) Three Km^R modules. They share a common right end that was constructed by insertion of a Bam HI linker into the Fnu4H I site that lies across the junction between Km^R and IS*903*-R. The left end of module B is a Bam HI linker at the Ava II site of IS*903* (position 945) (*13*). Module C contains exactly 20 bp from the right end of IS*903*-L; it is separated from the terminal Eco RI or Bam HI sites by 40 bp of non-IS*903* sequences. Module D contains a Bam HI linker at position 1082 of Tn*903*; it was derived by Bal31 digestion into the left end of module C. (E and F) Derived by Bal31 digestion from the Sal I site that lies to the right of IS*903*-R; deletions were terminated with Eco RI linkers, and end points were determined by restriction endonuclease and DNA sequence analyses. The deletion in module E stops 10 bp short of the right (outer) end of IS*903*-R while that in module F removes the terminal 45 bp of IS*903*-R (but leaves the coding frame intact).

IS*903* terminal inverted repeat was joined to new target DNA from the *galK* region of the plasmid (data not shown). As can be seen from Fig. 3 the deletions extended to various end points. This is consistent with the view that IS*903* does not have any marked specificity for its target site (*13, 15*).

Analysis of plasmid DNA isolated from Gal^R survivors from cells containing the transposase-defective control plasmid pTW76 showed that the background level of survivors was due in part to insertions of IS*1* and IS*2* into the *galK* region. Some plasmids gave the same restriction map as the parental pTW76 and proved to retain an active *galK* gene; we suspect that a chromosomal mutation rendered their hosts resistant to galactose.

Several replicative models for transposition predict that both deletions and inversion-insertions are the result of an intramolecular cointegrate process (*18–20*). Whether the outcome is deletion or inversion-insertion is determined only by the orientation of the target site when it interacts with the transposon. By demanding retention of kanamycin resistance along with acquisition of galactose resistance we can select for IS*903*-mediated events that inactivate *galK* without deleting the intervening DNA. We have analyzed 20 Km^R Gal^R clones from strains with pTW75 or with the structurally similar plasmid pTW90 (see Fig. 4). None of these contained inversion-insertions, although five did contain a second insertion of IS*903*; the rest were not IS*903*-mediated events.



	Frequency Ap^R Gal^R	Km^R/Ap^R
pTW75	2.5×10^{-4}	0.01 – 0.05
pTW76	2.0×10^{-6}	1

Fig. 2. IS*903* mediates deletions. The structures of pTW75 and pTW76 are as shown. pTW75 is a derivative of pBR322 that contains the Tn*903* modules A and B (see Fig. 1) inserted between the Eco RI and Bam HI sites (pBR322 sequences are shown as shaded blocks). The *galK* segment (wavy line) is the entire 2.5-kb segment of pKG1900 (*34*) that runs from the Eco RI site to the *gal*-pBR322 fusion point (the Pvu II site of pBR322); this region contains the *galK* gene of *E. coli* under control of the *gal* promoter. pTW76 is identical to pTW75 except that the 520-bp Pvu II fragment from within IS*903* has been deleted to inactivate the transposase. Plasmids were introduced into the host strain NG135 (K12 *gal*ΔS165 *recA56 strA*) by transformation. Rearrangements that inactivated the *galK* gene were selected by plating about 10^7 cells from a series of independent overnight cultures onto LB-agar plates containing 1 percent galactose and carbenicillin (40 μg/ml); plates were incubated at 42°C. The frequencies of Ap^R Gal^R indicate the proportion of cells that survive, averaged from four to six independent cultures. *Km^R/Ap^R* indicates the proportion of Ap^R Gal^R clones that retained resistance to kanamycin.

Deletion Formation Requires Both Ends of IS*903*

Formally, the result of an IS-mediated deletion is a new junction between one end of the IS and the target DNA; the other IS end remains at its original location. We have constructed plasmids to test whether the deletion-distal end of IS*903* plays a role in formation of deletions adjacent to the other end. Deletion formation from the left end of IS*903*-L was tested with the plasmids pTW105 and pTW106. Plasmid pTW106 differs from pTW105 only in that it contains an IS*903* terminus at its right end (Fig. 4A). Rearrangements resulting in galactose resistance occur in pTW106 at normal frequency (similar to pTW75), but are reduced to background levels in pTW105 (Fig. 4A). Restriction analysis of plasmid DNA from Gal^R clones showed, as expected, that pTW106 was yielding a high frequency of IS*903*-mediated deletions, but that loss of the deletion-distal IS end prevented formation of similar deletions in pTW105.

Analogous experiments with a second pair of plasmids, pTW90 and pTW88, gave similar results (Fig. 4B), showing that the left end of IS*903*-L is required for the normal high frequency of deletion formation from the right end. In the case of pTW88, however, restriction analysis of plasmid DNA from Gal^R clones showed that a few deletions joining the right end of IS*903* to *galK* had occurred. Since both ends of IS*903* are clearly required for a high frequency of deletion formation, we suspect that an alternative sequence is fulfilling the role of the deletion-distal terminus in the low frequency formation of IS*903*-mediated deletions in pTW88. One potential sequence with partial homology to the IS*903* end resides within IS*903* itself at position 931 to 939 (*13*); this 9-bp sequence is identical to position 2 to 10 in the terminal inverted repeat and in pTW88 has the correct (inverted) orientation relative to the deletion-proximal end. Machida *et al.* (*21*) showed that IS*102*, a transposon highly homologous to IS*903* (*22*), can occasionally use sequences with partial homology to the terminal inverted repeat as alternative ends in transposition.

For both pTW105 and pTW88 we investigated whether replacing an IS*903* end restored deletion formation to its normal high frequency. Using the module that contains just 20 bp from the right end of IS*903*-L, we constructed the plasmids pTW107 and pTW110 (see Fig. 4). In both cases the recombinogenic activity of IS*903* was completely regained. We analyzed plasmid DNA from more than 100 Gal^R clones derived from pTW106 and pTW107; all contained deletions adjacent to IS*903*; none contained an inversion-insertion.

It is noteworthy that deletion formation in pTW90 is consistently between one-third and one-sixth as efficient as that in either pTW75 or pTW110, even though the IS*903* sequences in the two are identical; the only difference between pTW90 and pTW75 is the DNA sequence between the left end of IS*903*-L and the Eco RI site of pBR322. Previous analyses of the behavior of IS*903*-R (from which the left IS end of pTW90 was derived; see Figs. 1 and 4) also suggest that the right end of this IS has reduced transpositional activity (*13, 16*). We conclude that the sequence immediately adjacent to an IS end can have a strong influence on its transpositional activity.

Both Products of an IS*903*-Mediated Deletion Can Be Recovered

That IS-mediated deletion formation requires both IS ends can be explained in two ways. Initiation of all transpositional activity may require the formation of a complex of two IS ends and the transposase, even though only one of the ends may subsequently be joined to the target (see Fig. 5A). Alternatively, both IS ends may actually take part in the recombinational process and be joined to target sequences (Fig. 5B). The second explanation is an alternative way of saying that deletions occur by an intramolecular cointegrate process, rather than by a direct simple insertion process.

A prediction arising from the second hypothesis is that deletions should be replicative and reciprocal, releasing the deleted DNA as a circle with a copy of the IS joining the two ends of the deleted segment. Testing this prediction requires the construction of a DNA substrate that contains a replication origin between the IS end and the target DNA. We have achieved this through the simple expedient of isolating IS*903*-mediated cointegrates between pTW75 and the conjugative plasmid pOX38, a derivative of the *E. coli* F factor that lacks transposons

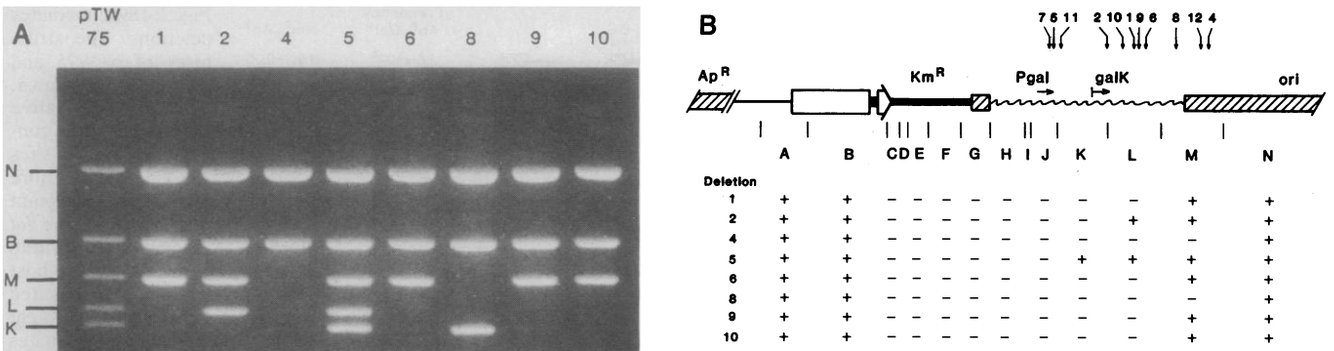


Fig. 3. Analysis of Gal^R deletions of pTW75. (A) A 1.3 percent agarose gel displaying the products of Taq I digestion of pTW75 and several deletions. Two fragments comigrate at band G in the pTW75 digest but there is only one at this position in lanes 1 to 10. Also the new IS*903-galK* junction fragment of deletion 4 comigrates with band E, while that of deletion 10 comigrates with band H. (B) Map of pTW75 showing Taq I cleavage sites and deletion end points. For each of the deletions analyzed in (A) the presence (+) or absence (−) of specific restriction fragments is indicated below the map.

(*23*). Cointegrates are readily isolated by selecting for the conjugative transfer of pTW75 from a host strain containing pOX38 into an F$^-$ recipient. Since pTW75 is not normally mobilizable, the only way it can be transferred is through formation of a recombinant with pOX38. As diagrammed in Fig. 6, we have shown that such recombinants are cointegrates with two copies of IS*903* (*5*).

In attempting to determine whether a single deletion event generates two recoverable products, let us consider the process shown in Fig. 6. A deletion into *galK* mediated by the IS*903* proximal to the ampicillin resistance (Ap^R) gene [but not one mediated by the IS proximal to the kanamycin resistance (Km^R) gene] will generate two products, each with a separate origin of replication. However, since distinguishable plasmids with the same replication system are incompatible, each product will be incompatible with the parent double-origin cointegrate. A cell in which a deletion has just been formed will contain several copies of the parent cointegrate (*24*) and one copy of each product replicon. How can we maximize the chances of recovering those cells in which both products have survived? We reasoned that because of its low potential copy number, the pOX38-Km^R product would stand the least chance of survival. In fact, since the copy number of the parent plasmid already exceeds that normally allowed by the F replication control system, pOX38-Km^R will rarely replicate or segregate away from its parent replicon. Therefore, to recover those rare cells that have retained the pOX38-Km^R product replicon requires selecting for retention of kanamycin resistance at the same time as selecting for acquisition of galactose resistance (*25*). What of the other (potential) reciprocal product, the pBR322-Ap^R replicon? Although the pBR322 replication system increases the copy number of the pOX38::pTW75 parent plasmid above that of pOX38, it remains considerably lower than the copy number of pBR322 itself (or of derivatives such as pTW75). Since the total number of pBR322 replication origins in the parental strain will be less than the number in a strain that contained only pTW75, it seemed likely that

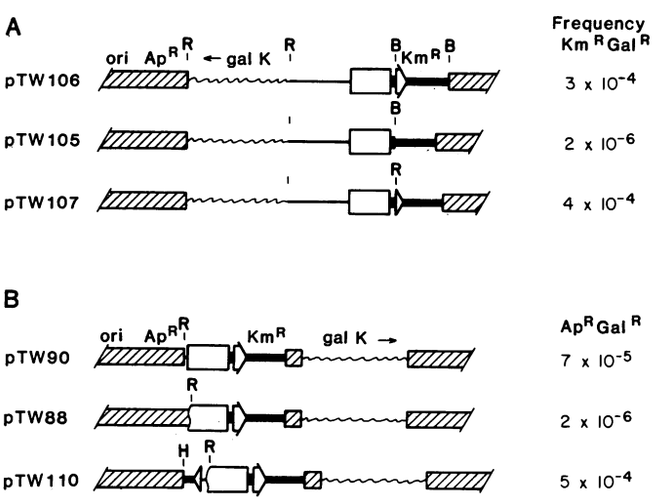


Fig. 4. Deletion formation requires both ends of an IS. (A) Deletions from the left end of IS*903*-L. pTW106 contains modules A and B (see Fig. 1) inserted between the Eco RI and Bam HI sites of pBR322. The *galK* segment is the 2.6-kb Eco RI–Tth111 I fragment from pKG1800 (*34*) and is inserted into the Eco RI site with the direction of *galK* expression as shown. pTW105 is like pTW106 but contains the Km^R module D (Fig. 1) and hence has no IS*903* terminal inverted repeat at the IS-Km^R junction. pTW107 is like pTW106 but contains the Km^R module C; it has 20 bp from the right end of IS*903*-L at the IS-Km^R junction. Frequencies of Gal^R derivatives were determined as described in Fig. 2. (B) Deletions from the right end of IS*903*-L. pTW90 is like pTW75 (Fig. 2) except that the left portion of the IS*903* and all the DNA adjacent to the Eco RI site of pTW75 has been replaced with module E; the translational reading frame and both ends of the IS are retained. pTW88 is like pTW90 but contains module F in place of E (Fig. 1); pTW88 therefore does not have an intact terminal inverted repeat at the IS-Ap^R junction. pTW110 was derived from pTW88 by inserting the 720-bp Eco RI–Hind III segment from module C into the Eco RI site, in the orientation shown, to provide the left-terminal inverted repeat.

a pBR322-Ap^R replicon formed in a deletion would stand a good chance of being replicated and, therefore, of being segregated into any cell that had captured a pOX38-Km^R product. Events that inactivate *galK* other than IS*903*-mediated deletions will not remove the pBR322 origin of pOX38::pTW75. Such plasmids will be likely to compete with the parent replicon on an approximately equal basis and might therefore predominate in the Gal^R Km^R progeny. To detect possible reciprocal deletions among the selected clones we therefore screened plasmid DNA from individual colonies for the presence of a small multicopy plasmid.

Gal^R Km^R clones from NG135 carrying pOX38::pTW75 cointegrates were selected as before. Such clones arose at a frequency of about 2×10^{-4} for 12 different cointegrates tested. Electrophoretic analysis of plasmid DNA showed that some Gal^R Km^R colonies contained both a small high-copy plasmid and a large low-copy plasmid (lanes 1 to 3 in Fig. 6). A total of 145 colonies were analyzed from 12 different cointegrates, and in six colonies we detected two plasmids of the size and copy number expected for a reciprocal deletion.

All six pairs of plasmids have been extensively analyzed to determine whether, in fact, each pair was derived from a single event. The six large low-copy plasmids had the following properties. They could transfer Km^R at high frequency in bacterial matings but had lost Ap^R and *galK* activity. Molecular analyses demonstrated that each retained two copies of IS*903*. For five of the six, this was shown by first subcloning into pNG16 (*13*) an Eco RI fragment that contained the entire pTW75-derived DNA segment (the single Eco RI site of pTW75 is lost from the parental pOX38::pTW75 in the deletion formation). In digests of the subclones with Taq I and with Pvu II the internal IS*903* fragments (Pvu II, 520 bp or Taq I, 950 bp) were present in twice the molar amount of the other fragments. Digestion of the parental cointegrates with Bam HI releases a 1.1-kilobase (kb) and a 7.5-kb fragment from the pTW75 region of each plasmid. Bam HI digestion of the Km^R deletion products released the expected 1.1-kb fragment (diagnostic of the Km^R region) and a new fragment of 2 to 3 kb that contained the new IS-*galK* junction. The size of this new fragment is indicative of the deletion end point within *galK* and therefore differs among the six plasmids analyzed.

The six corresponding low molecular weight, high-copy plasmids had the following properties. They conferred resistance to ampicillin, but not to kanamycin, and had lost *galK* activity. Each contained a single copy of IS*903*, since Taq I digests produced the diagnostic 950-bp fragment in amounts equimolar to the other fragments. The Taq I digests showed that the general structure of the multicopy plasmids was the same as that of the Km^S deletion products of pTW75 (Fig. 3) and the deletion end points could be precisely determined in the same manner. In all cases the extent of the *galK* region carried by a multicopy plasmid was complementary to that carried by the corresponding pOX38-Km^R deletion product, so that together each pair of plasmids contained one complete set of *galK* sequences.

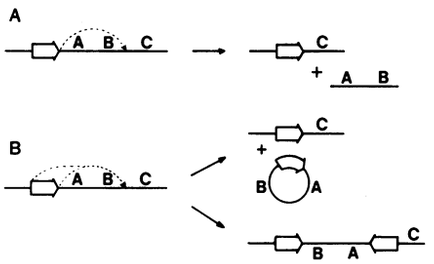


Fig. 5. Deletion formation by an IS. (A) Transpositional recombination involving just one IS end. The actual mechanism could be conservative, involving double-strand cuts at the IS end and the target (*32*), or it could be replicative, involving transfer of a single strand of the IS to the cut target site (*19, 31*). (B) Transpositional recombination involving both IS ends. For a deletion to be obtained, this must be a replicative process (otherwise the left IS junction will be broken). This is the intramolecular cointegrate pathway [for details see (*18*) and (*19*)] and, depending only on the orientation of the target relative to the IS ends, will result in a deletion (top) or an inversion-insertion.

The true test of reciprocity in formation of these deletions is to show by DNA sequence analysis that the new IS*903-galK* junctions of both products occur at the same site. For three pairs of deletion products we have sequenced these junctions (the regions indicated by the bars in Fig. 6) and the results are shown in Fig. 7. Each pair of plasmids shares a common 9-bp target sequence at the IS*903-galK* junction. The remaining *galK* sequences are mutually exclusive, with the low-copy plasmid containing the beginning of the *galK* gene and the multicopy plasmid the end. The shared 9 bp was expected since Tn*903* causes duplication of a 9-bp target sequence during intermolecular transposition (*15*).

It is clear from these results that deletion formation by IS*903* is a reciprocal recombination event involving both ends of the IS. Since the parental cointegrate contains two copies of the IS, and the two replicons resulting from the deletion together contain a total of three (two on

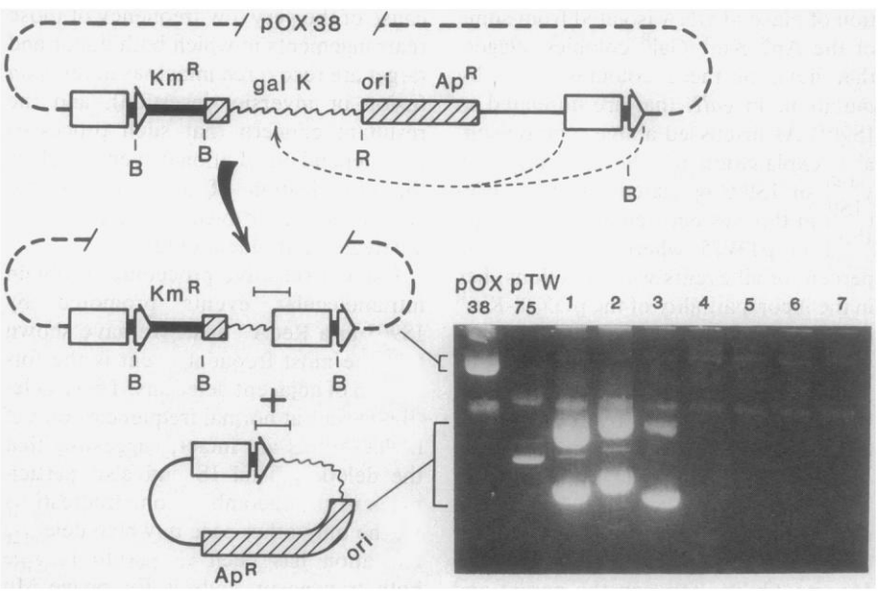


Fig. 6. Structure of a pOX38::pTW75 cointegrate and predicted deletion products. The cointegrate (top) is a fusion of pOX38 (dashed lines) and pTW75 that contains one copy of IS*903* at each junction between the parent replicons. Deletion from the right, Ap^R-proximal IS*903* into *galK* (but not from the Km^R-proximal IS*903*) is predicted to create the two replicons pOX38-Km^R and pBR322-Ap^R shown below if deletion occurs by a replicative cointegrate pathway. Displayed on the right are plasmid DNA's from seven independent Gal^R Km^R clones analyzed by electrophoresis on a 0.7 percent agarose gel. The two left lanes show pOX38 and pTW75; lanes 1 to 3 show DNA of three clones that contained both high- and low-copy plasmids (reciprocal products of a deletion event); lanes 4 to 7 show DNA from four clones that contain only a low-copy plasmid. The bars (⟼) indicate the regions of the pOX38-Km^R and pBR322-Ap^R replicons that were sequenced (see Fig. 7).

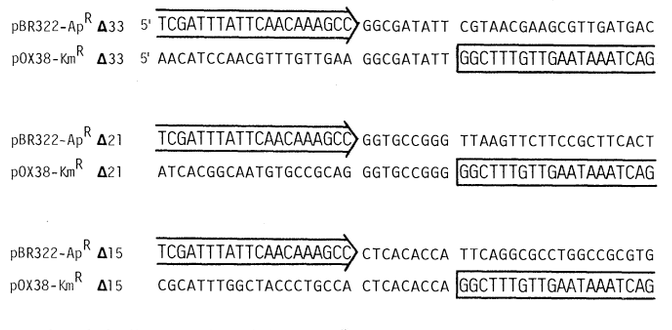


Fig. 7. DNA sequences of reciprocal products of IS*903* deletions. The sequences of one DNA strand across the IS*903-galK* junction of the two products from three separate deletion events (Δ33, Δ21, and Δ15) are shown. The IS*903* terminal sequences are shown in large type and are boxed; the arrowhead indicates the right (Km^R-proximal) end of IS*903*-L and corresponds to the arrowhead in Fig. 6. The *galK* coding strand is shown in smaller type. The sequences are aligned to show the 9-bp *galK* target sequence that occurs at each junction. Sequences were determined by the dideoxy chain termination method of Sanger *et al.* (*35*), with restriction fragments cloned into the single-strand M13 vectors mp8 or mp9 (*36*).

the pOX38-Km^R replicon, one on the pBR322-Ap^R replicon), it also clearly involves replication of the IS element that mediates the event.

As mentioned above, only six of 145 Km^R Gal^R colonies screened yielded two separate replicons. We have carried out a preliminary analysis of the events that do not result in the production of two replicons. Analysis of 12 clones that contained only a large low-copy plasmid showed that all retained both the Km^R selected marker and the unselected Ap^R locus. All 12 transferred both Ap^R and Km^R at high frequency with 100 percent linkage but had lost *galK* activity. This proves that these 12 Gal^R clones did not arise by a deletion adjacent to IS*903*. A preliminary analysis by restriction digestion of plasmid DNA isolated from some of the Ap^R Km^R Gal^R colonies suggest that most of these colonies arose by mutations in *galK* that are unrelated to IS*903*. As discussed above, one reasonable explanation for the relatively low yield of IS*903*-mediated adjacent deletions in this system (relative to the high yield in pTW75 where more than 95 percent of all events were deletions) lies in the incompatibility of the pOX38-Km^R deletion product with the parental cointegrate.

An important question is whether all IS*903* deletions occur by a reciprocal process. Unfortunately, the problems associated with plasmid incompatibility make the system that we have used to demonstrate reciprocity a poor one for addressing this question quantitatively. Incompatibility between the parent and product replicons could influence the results in three ways. First, it may reduce the frequency of deletion events recovered (as we argue above); second, it may result in the loss of one product from a reciprocal event, suggesting possible nonreciprocity; and third, it may result in stronger selection against recovery of nonreciprocal deletions than against reciprocal deletions (*26*). With these limitations in mind we have looked among Gal^R Km^R clones for possible nonreciprocal deletion products. Deletions into *galK* that retain Km^R must have originated from the Ap^R-proximal IS*903* and, if nonreciprocal, should lose Ap^R. We have screened 50 Gal^R Km^R clones and found just one that had lost Ap^R.

Conclusions and Discussion

It has proved difficult to obtain conclusive evidence that any genetic rearrangements mediated by transpositional recombination of an IS element involve duplication of the IS. This is partly because of the very low frequency of those rearrangements in which both donor and target are recovered intact (as in replicon fusion or inversion-insertion), and the resultant concern that such processes may depend on additional events such as the dimerization of a plasmid donor (which can occur even in RecA$^-$ cells at a detectable frequency) (*27*).

Using a selective procedure to obtain intramolecular events promoted by IS*903* in a RecA$^-$ host, we have shown that the most frequent event is the formation of adjacent deletions. These deletions occur at normal frequencies only if both IS ends are intact, suggesting that the deletion-distal IS end also participates in the recombination. Interestingly, the only other case in which deletion formation has been shown to require both transposon ends is for phage Mu (*28*), a transposon that clearly has a high frequency of replicative cointegrate formation (*29*). We were able to recover two products from a single deletion event by giving the deleted segment a replication origin of its own. Each product was a separate replicon with a copy of the mediating IS and the 9-bp target sequence. While these results do not prove that all IS*903* recombinational events are replicative, they do indicate that a replicative mechanism is a major pathway of transpositional recombination. Determining whether simple insertion of an IS is a replicative process will probably have to await a biochemical characterization of the recombination.

Our findings provide strong support for the idea that IS-mediated deletions are formed by an intramolecular cointegrate process as was first suggested by Shapiro (*18*). However, these findings do not distinguish between molecular models that propose simultaneous ligation of both transposon ends to the target (*18, 30*), and those that propose that these ligations are separated in time, with transposon replication interposed (*19, 20, 31*). If the most frequent intramolecular rearrangement mediated by an isolated IS element occurs by a cointegrate process, then our results raise two paradoxes. First, in plasmids such as pTW75, why do we not observe inversion-insertions at about the same frequency as adjacent deletions, since both are predicted to be alternative products of the same process (see Fig. 5B)? Perhaps in our assay, in which the target is separated from the IS by only about 3 kb, its orientation relative to the IS ends is determined by topological considerations or by other plasmid properties (such as direction of replication). Second, in intermolecular transposition of IS*903*, simple insertions appear to be favored over cointegrates by a factor of about 100, the reverse of the intramolecular results (*5, 16*). Perhaps simple insertion is normally a conservative process involving excision of the IS and consequent loss of its parent replicon (*32*), so that intramolecular simple insertions would be suicidal.

References and Notes

1. *Cold Spring Harbor Symp. Quant. Biol.* **45** (1981); J. A. Shapiro, *Mobile Genetic Elements* (Academic Press, New York, 1983).
2. N. Kleckner, *Annu. Rev. Genet.* **15**, 341 (1981).
3. N. D. F. Grindley, *Cell* **32**, 3 (1983).
4. R. Gill, F. Heffron, G. Dougan, S. Falkow, *J. Bacteriol.* **136**, 742 (1978).
5. N. D. F. Grindley and C. M. Joyce, *Cold Spring Harbor Symp. Quant. Biol.* **45**, 125 (1981).
6. D. J. Galas and M. Chandler, *J. Mol. Biol.* **154**, 245 (1982).
7. B. J. Hirschel, D. J. Galas, M. Chandler, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4530 (1982).
8. D. E. Berg, *ibid.* **80**, 792 (1983).
9. G. Cornelis and H. Saedler, *Mol. Gen. Genet.* **178**, 367 (1980); N. Kleckner and D. G. Ross, *J. Mol. Biol.* **144**, 215 (1980).
10. H. Saedler, G. Cornelis, J. Cullum, B. Schumacher, H. Sommer, *Cold Spring Harbor Symp. Quant. Biol.* **45**, 93 (1981).
11. J. C. Liebart, P. Ghelardini, L. Paolozzi, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4362 (1982).
12. E. Ljunquist and A. I. Bukhari, *ibid.* **74**, 3143 (1977).
13. N. D. F. Grindley and C. M. Joyce, *ibid.* **77**, 7176 (1980).
14. A. Oka, H. Sugisaki, M. Takanami, *J. Mol. Biol.* **147**, 217 (1981).
15. A. Oka, N. Nomura, K. Sugimoto, H. Sugisaki, M. Takanami, *Nature (London)* **276**, 845 (1978).

16. T. A. Weinert, N. A. Schaus, N. D. F. Grindley, unpublished results.
17. H. Ohtsubo and E. Ohtsubo, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 615 (1978); A. Bernardi and F. Bernardi, *Gene* **13**, 103 (1981).
18. J. A. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1933 (1979).
19. R. M. Harshey and A. I. Bukhari, *ibid.* **78**, 1090 (1981).
20. D. J. Galas and M. Chandler, *ibid.*, p. 4858.
21. Y. Machida, C. Machida, E. Ohtsubo, *Cell* **30**, 29 (1982).
22. A. Bernardi and F. Bernardi, *Nucleic Acids Res.* **9**, 2905 (1981).
23. M. S. Guyer *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **45**, 135 (1981).
24. Since the parent cointegrate contains the replication system of the multicopy plasmid pBR322, its copy number is intermediate; it is higher than that of pOX38 which, like the F factor, is one to two copies per cell, but is lower than that of pBR322 because of its larger size (about 60 kilobases).
25. The selection for Km^R also prevents us from recovering deletions from the Km^R-proximal IS*903* into *galK* since all of these would lose kanamycin resistance (and none could give two recoverable products whether deletions were reciprocal or not).
26. This is because the pBR322-Ap^R replicon resulting from a reciprocal deletion may moderate replication of the parent molecule and therefore improve the chances of survival of the pOX38-Km^R deletion product.
27. R. A. Fishel, A. A. James, R. Kolodner, *Nature (London)* **294**, 184 (1981).
28. M. Faelen and A. Toussaint, *J. Bacteriol.* **136**, 447 (1978); a similar claim has been made for IS*1* (*10*), however, it seems likely that the IS*2* insertion into the IS*1* end has actually disrupted transposase expression and may well have left the terminal sequence intact.
29. R. M. Harshey, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2012 (1983).
30. A. Arthur and D. Sherratt, *Mol. Gen. Genet.* **175**, 267 (1979).
31. N. D. F. Grindley and D. J. Sherratt, *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1257 (1979).
32. D. E. Berg, in *DNA Insertion Elements, Plasmids, and Episomes*, A. I. Bukhari, J. A. Shapiro, S. L. Adhya, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), p. 205.
33. L. Guarente, G. Lauer, T. M. Roberts, M. Ptashne, *Cell* **20**, 543 (1980).
34. K. McKenney, H. Shimatake, D. Court, U. Schmeissner, C. Brady, M. Rosenberg, in *Gene Amplification and Analysis*, J. C. Chirikjian and T. S. Papas, Eds. (Elsevier-North Holland, Amsterdam 1981), vol. 2, p. 383.
35. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
36. J. Messing and J. Vieira, *Gene* **19**, 269 (1982).
37. We thank G. Hatfull and C. Joyce for discussions; A. Weiner, C. Joyce, and T. Platt for critically reading the manuscript and B. Newman for some plasmid constructions. This work was supported by NIH grant GM28470.

Translocations Among Antibody Genes in Human Cancer

Philip Leder, Jim Battey, Gilbert Lenoir
Christopher Moulding, William Murphy, Huntington Potter
Timothy Stewart, Rebecca Taub

Geneticists have become increasingly aware of the fact that genes move, that they change position within and between chromosomes, and that they thus alter their relationships to one another with important regulatory and structural consequences. One dramatic example of this occurs as part of the process by which the immunoglobulin (Ig) genes are normally formed by joining subgenomic segments of DNA in various combinations to create the enormous diversity reflected by the immune system (*1*). Another example, by no means normal and with an apparently more sinister outcome, is represented by the somatic translocations that consistently accompany certain human neoplasms (*2*). In recent months, these two phenomena have been physically linked by studies showing that the human cellular oncogene c-*myc* is joined to one of the Ig loci by a translocation that characteristically accompanies a human malignancy called Burkitt lymphoma (*3–7*). Similar results have been obtained in mouse plasmacytomas (*8–13*).

The juxtaposition of these two rather well characterized genetic systems allows us to address two important—if poorly understood—questions: First, what are the molecular genetic consequences of these translocations and how do they alter the normal function and control of these genes? And second, how does their interaction contribute to the process of malignant transformation? While work in a number of laboratories is still in its earliest stages, certain observations already allow us to evaluate the most obvious models and to advance others. Our purpose here will be to consider briefly the normal Ig and *myc* genes before turning to specific molecular aspects of certain Burkitt lymphoma translocations and the early answers they bring to the questions we have posed.

Associations Between Chromosomal Translocations and Neoplasia

The close association between specific chromosomal translocations and certain human neoplasms is well established (*2*). Such translocations are of two types: constitutional, that is, those carried by each of the individual's cells; and somatic, those that arise in a particular cell and are carried by its neoplastic progeny. It is generally held that such constitutional translocations (and other chromosomal abnormalities) predispose an organism to the development of a malignancy, but require a second event, presumably another mutation, to consummate the malignant transformation (*14*). For example, in hereditary renal cell carcinoma there is a constitutional translocation involving chromosomes 3 and 8 [t(3;8)] that is associated with the development of renal cell carcinoma during the fourth decade of life (*15*). Somatic translocations, in contrast, presumably arise in a single somatic cell and contribute to the transformation of that cell and its progeny alone. It is not likely that either translocation is sufficient to accomplish the malignant transformation; it is quite likely that other critical events have occurred or will occur (*16*). Nevertheless, the tight association between particular malignancies and specific translocations [t(9;22) in chronic myelogenous leukemia and t(2;8), t(8;14), and t(8;22) in Burkitt lymphoma] makes a very strong, if circumstantial, argument that these translocations are causally related to the development of the neoplasm.

The discovery of oncogenes, originally noted as transforming genes carried or activated by retroviruses [see review (*16*)], combined with a growing understanding of transcriptional control mechanisms, provided the basis for two molecular models that could explain how a translocation might induce malignancy (*17*). A translocation could disturb the regulation of an oncogene, for example, by providing a new promoter region or some other control element that would activate the oncogene (*18*). Alternatively, it might alter the coding sequence of a gene, changing its protein product from a benign to a malignant form (*19*).

All the authors are associated with the Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115 except Gilbert Lenoir, who is at the International Agency for Research on Cancer, World Health Organization, 150 Cours Albert Thomas, 69372 Lyon, France.