IS*10* Promotes Adjacent Deletions at Low Frequency

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ABSTRACT

Some transposable elements move by a replicative mechanism involving cointegrate formation. Intramolecular cointegration can generate a product called an "adjacent deletion" in which a contiguous chromosomal segment adjacent to the transposon is deleted while the element responsible remains intact. Insertion sequence IS*10* is thought to transpose by a nonreplicative mechanism. In the simplest models, nonreplicative transposition cannot give rise to an adjacent deletion because an intrinsic feature of such transposition is excision of the IS element from the donor location. We report here that IS*10* can generate adjacent deletions, but at a frequency which is approximately 1/30th the frequency of transposition for the same element. We suggest that these deletions might arise either by nonreplicative transposition events that involve two IS*10* elements located on sister chromosomes or by aberrant nonreplicative events involving cleavage and ligation at only one end of the element.

MANY bacterial insertion sequences and transposons promote the occurrence of an event called an "adjacent deletion." Such rearrangements remove a contiguous stretch of DNA adjacent to one end of the transposon; the transposon itself remains intact at the new deletion junction (Figure 1A). For transposable elements that use a replicative transposition mechanism leading to cointegrate formation, adjacent deletions are understood to occur as the result of intramolecular cointegration events (SHAPIRO 1979; ARTHUR and SHERRATT 1979; WEINERT, SCHAUS and GRINDLEY 1983).

We wished to examine the formation of adjacent deletions by an insertion sequence, IS*10*, which is thought to transpose by a nonreplicative mechanism. IS*10* occurs at the ends of the composite transposon Tn*10*. During IS*10* or Tn*10* transposition, the element is excised cleanly from the donor site and inserted at a new site without extensive DNA replication (BENDER and KLECKNER 1986; BENJAMIN and KLECKNER 1989). Thus, for IS*10*, adjacent deletions should not occur as the consequence of intra-molecular transposition, because an obligatory step in the transposition reaction is complete excision of the element from the donor site. The situation for Tn*10* is more complicated (see below).

We show below that IS*10* does promote the formation of adjacent deletions, but at a frequency which is at least 30-fold lower than the frequency of IS*10* transposition. These observations provide further support for the view that IS*10* transposition does not involve cointegrate formation. We consider two models for how such deletions could arise. In one case, they occur by a variant of the standard nonreplicative transposition reaction which involves two IS*10* elements provided by sister chromosomes. An essentially similar model has been proposed independently by LICHENS-PARK and SYVANEN (1988) to account for rare IS*50*-mediated cointegrates. In the second case, adjacent deletions arise from aberrant nonreplicative events involving only one end of the element.

It should be noted that the full transposon Tn*10* does generate two types of intrachromosomal rearrangements which appear to be simple intramolecular variants of the standard transposition reaction. These rearrangements, called "deletion/inversions" and "adjacent deletions," probably arise by the interaction of Tn*10*'s two "inside" IS*10* ends with a target site located in adjacent sequences (Figure 1B, KLECKNER, REICHARDT and BOTSTEIN 1979; BENJAMIN and KLECKNER 1989). Since both IS*10* elements of Tn*10* are involved, these events are said to be *Tn10*-promoted rather than *IS10*-promoted. In fact, the consequence of a Tn*10*-promoted adjacent deletion is the same as that of one type of IS*10*-promoted adjacent deletion (compare Figure 1, B to A). The results presented here demonstrate that IS*10*-promoted adjacent deletions are much rarer than Tn*10*-promoted adjacent deletions and thus confirm that the adjacent deletions which arise in the presence of the full Tn*10* element are indeed *Tn10*- and not *IS10*-promoted.

MATERIALS AND METHODS

Media and enzymes: Bacteriological media were prepared as described by MILLER (1972) and FOSTER *et al.* (1981). When used, supplements were added at the follow-

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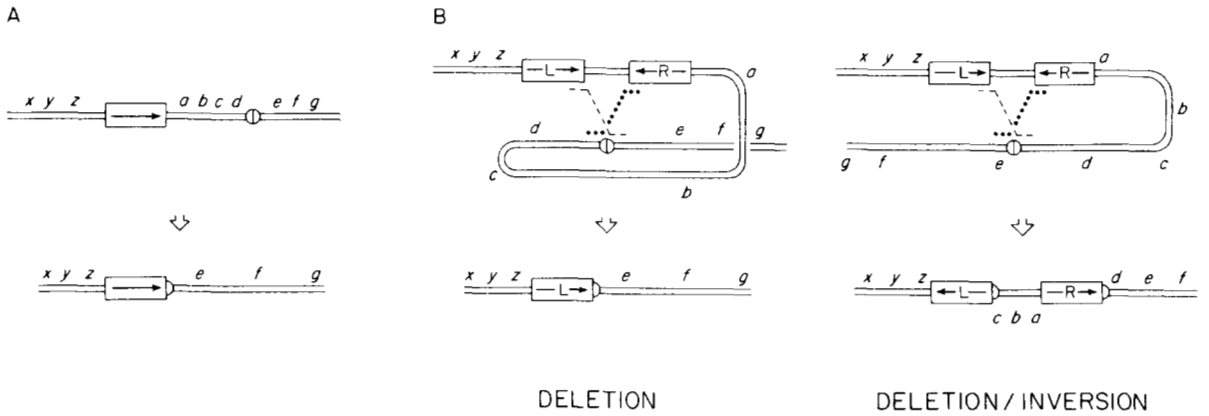

FIGURE 1.—A, Structure of an IS- or Tn-promoted adjacent deletion. B, Model for formation of Tn*10*-promoted adjacent deletions and deletion/inversions.

ing concentrations: amino acids, 50 μg/ml; ampicillin 100 μg/ml; chloramphenicol, 20 μg/ml; kanamycin sulfate, 50 μg/ml; nalidixic acid, 40 μg/ml; streptomycin sulfate, 150 μg/ml; tetracycline, 20 μg/ml. Amino acids and antibiotics were purchased from Sigma. Restriction enzymes were purchased from New England Biolabs.

Strains: Insertions of IS*10-kan* elements into *nadA* were isolated using an Hfr strain (Hfr Broda) that transfers the *nad/gal* region early. This strain was transformed with a multicopy plasmid carrying the IS*10* element of interest, pNK1166 (wild type) or pNK1168 (*HH104*). The transformed strain was mated with NK7025. Kan^R exconjugants were selected and screened for a nicotinamide requirement. Insertions that caused a Nad$^-$ phenotype were moved by P1 transduction into NK7434 to generate a *nadA*::IS*10 galE* strain. These transductants were examined by Southern blot analysis to confirm the presence of one and only one IS*10* element and to verify that all insertions were located in the same site in *nadA* and in the same orientation at that site. The *nadA*::Tn*10* insertion occurs at the same site, and with the same orientation of IS*10*-Right as the *nadA*::IS*10* insertions.

NK7434 is *galE leu2 trp3 his1 arg6 metB tonA tsx supE44 xyl7 mtl2 ΔlacZr1*. NK7435 is an isogenic strain that contains *nadA*::Tn*10*. NK7887 and NK7888 are isogenic strains that contain insertions of IS*10-kan* (ROBERTS *et al.* 1985) in the *nadA* gene. The insertion in NK7887 is wild type for IS*10* sequences and the insertion in NK7888 carries the IS*10* promoter mutation *HH104* (SIMONS *et al.* 1983) which increases expression of transposase. NK7025 is *lacΔX74 galOP38 StrR*.

For RecA$^-$ derivatives of NK7434 (used in Tables 2 and 3 and Figures 3 and 4), *recA56* was introduced by first transducing strains to Tet^R with P1 grown on NK5991 (W3110 *srl*::Tn*10*), and then transducing to Srl$^+$RecA$^-$ with P1 grown on NK5830 = *ΔlacproXIII recA56* Arg$^-$ Nal^R Rif^R/F′ *lac pro laci^{Q}L8*. P1 lysates were prepared as described in MILLER (1972).

Selection of Gal^R revertants and assaying of transposition: Gal^R revertants were selected as described in ROBERTS *et al.* (1985). Single Gal^R revertant colonies were picked and tested for other phenotypes. Colonies were scored as AroG$^-$ if they failed to grow on minimal plates containing tryptophan and tyrosine but lacking phenylalanine. Colonies were scored as Pgl$^-$ if they stained blue with iodine after growth on minimal plates containing maltose as the sole carbon source (KUPOR and FRAENKEL 1969).

Transposition (Table 3) was tested using the "mating out" assay of FOSTER *et al.* (1981). pOX38::Tn*9* (WAY *et al.* 1984) was mated into each strain to be tested. A mating was then done between these donors and a suitable recipient strain, NK7005 (= F$^-$ λ^R Arg$^-$ *recA56* Nal^R Rif^R *ΔlacproXIII*). Total exconjugants were measured as Nal^R Cam^R colonies, and transposition events were measured as Nal^R Kan^R colonies.

Southern blot analysis: Southern blots were performed according to MANIATIS, FRITSCH and SAMBROOK (1982) using nick translated pNK82 as probe. pNK82 (FOSTER *et al.* 1981) contains all of IS*10*-Right plus adjacent Tn*10* loop material extending from the inner end of IS*10*-Right to the *Eco*RI site in Tn*10*. pNK82 also contains part of the *Salmonella typhimurium hisG* gene, but the IS*10* sequences are the only sequences that hybridize to DNA of *Escherichia coli* strains containing IS*10* under the conditions used. Chromosomal DNA was prepared as described in RALEIGH and KLECKNER (1984) and SHEN, RALEIGH and KLECKNER (1987).

RESULTS

Genetic evidence for IS*10*-promoted deletions: We have previously examined the formation of Tn*10*-promoted adjacent deletions using a Tn*10* element inserted in the *nadA* gene of a GalE$^-$ *E. coli* strain. GalE$^-$ strains fail to grow in the presence of galactose (are "galactose-sensitive") because they accumulate a toxic phosphorylated intermediate in galactose catabolism. Galactose-resistant (Gal^R) revertants of the GalE$^-$ strains arise spontaneously due to inactivation of the *galK* gene, whose product is needed for conversion of galactose to the toxic intermediate (*e.g.*, ADHYA and SHAPIRO 1969).

The *nadA* locus is closely linked to the *gal* operon (Figure 2). The frequency of Tn*10*-promoted rearrangements is high enough that the presence of a *nadA*::Tn*10* insertion increases the frequency of Gal^R revertants five- to tenfold above the spontaneous level (ROBERTS 1986). The vast majority of such revertants (>80%) are adjacent deletions as shown by physical and genetic analysis (ROBERTS, 1986; our unpublished results): essentially all of them (98%) have lost the *aroG* gene located between *nad* and *gal* (Figure 2). Furthermore, most of these Aro$^-$ deletions (95%)

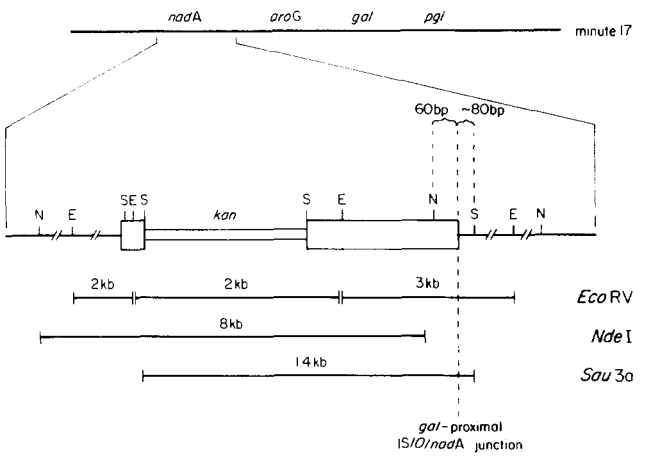

FIGURE 2.—Structure of the *nadA/gal* region of *E. coli* K12. Top line: order of markers in minute 17 of the chromosome. The total distance from *nadA* to *pgl* is approximately 15 kb. Bottom line: restriction map of the *nadA*::IS*10-kan* insertion and surrounding regions, not drawn to scale. Relevant fragments and fragment sizes are shown. E = *Eco*RV; N = *Nde*I; S = *Sau*3a.

extend beyond *gal* into or beyond the *pgl* gene, and about 25% extend as far as *bio* and *uvrB* which are located a minute away (ROBERTS 1986).

We have now used the *nadA/gal* system to assay for adjacent deletions promoted by an individual IS*10* element. A single IS*10* element marked with a kanamycin-resistance determinant (IS*10-kan*) was introduced into the *nadA* locus. Isogenic constructs were made that contained either a wild type IS*10-kan* element or a mutant IS*10-HH104-kan* element which transposes at 10 to 40 times the rate of the wild type element (ROBERTS *et al.* 1985).

Genetic analysis of these constructs provides strong evidence that IS*10-HH104-kan* generates adjacent deletions of the classical type:

1. The *nadA*::IS*10-HH104-kan* insertion increases the frequency of Gal^R revertants about 20-fold (Tables 1 and 2). The formation of Gal^R revertants by this element is not dependent on *recA* function (Table 2).

2. The vast majority of Gal^R revertants isolated either in RecA$^+$ (Table 1) or RecA$^-$ strains (data not shown) have arisen as the consequence of a deletion: roughly 85% are AroG$^-$. Furthermore, the same spectrum of deletions is observed in this case as was observed previously for *nadA*::Tn*10*-promoted adjacent deletions; nearly all of the AroG$^-$ deletions are also Pgl$^-$ (Table 1).

3. Formation of Gal^R revertants by *nadA*::IS*10-HH104-kan* requires IS*10* transposase. In both RecA$^+$ and RecA$^-$ hosts, the frequency of revertants is reduced to the *nadA*$^+$ level upon introduction of a multicopy plasmid that specifies very high levels of the IS*10*-encoded antisense RNA, RNA-OUT (Table 2). RNA-OUT specifically inhibits transposase expression by pairing with the 5′ end of its mRNA and thus blocking ribosome binding (SIMONS and KLECKNER 1983; reviewed in KLECKNER 1988). The frequency of Gal^R revertants is reduced to a lesser extent by an isogenic plasmid that specifies somewhat lower levels of antisense RNA (Table 2). Furthermore, both antisense RNA plasmids preferentially reduce the AroG$^-$ class of Gal^R revertants (data not shown), as expected if all of these deletions and only some of the AroG$^+$ revertants result from IS*10*-promoted events. In these experiments, a *nadA*::Tn*10* insertion was included as a control. This insertion increases the frequency of Gal^R five- to tenfold, independent of RecA function, and with inhibition by RNA-OUT (Table 2).

4. All Gal^R revertants (90/90 for the experiment in Table 1) are kanamycin resistant, suggesting that they have retained (at least part of) the marked IS*10* element. Furthermore, the marked IS*10* element has retained its overall genetic integrity as shown by transposition assays. Four Aro$^-$ Gal^R revertants were tested and found to exhibit transposition frequencies identical to that of the parental insertion (Table 3).

The wild-type *nadA*::IS*10* element also appears to promote adjacent deletions. This insertion increases the overall frequency of Gal^R revertants only marginally (by 10%, Table 1). However, it substantially increases the frequency of Gal^R AroG$^-$ revertants which are diagnostic of IS*10*-promoted deletions, from 0/90 to 9/90. Similar results have been observed in several experiments besides the one shown here.

Physical evidence for IS*10*-promoted adjacent deletions: The Gal^R Aro$^-$ revertants arising in the presence of *nadA*::IS*10-HH104-kan* have the physical structure expected for IS*10*-promoted adjacent deletions. Specifically, the IS*10/nadA* junction farthest from *gal* is present intact, but the IS*10/nadA* junction closest to *gal* has been altered.

DNAs from 10 independent Gal^R Aro$^-$ revertants of the RecA$^-$ GalE$^-$ *nadA*::IS*10-HH104-kan* strain were digested with appropriate restriction enzymes and subjected to Southern blot analysis using a probe that contains all of wild type IS*10* (without the kanamycin marker) and some additional irrelevant sequences (MATERIALS AND METHODS).

DNAs were first analyzed following cleavage with *Eco*RV. As shown in Figure 2, cleavage of the parent strain with this enzyme generates three fragments containing IS*10* sequences: a right (*gal*-proximal) junction fragment of 3 kb, a left (*gal*-distal) junction fragment of 2 kb, and a central fragment of 2 kb; the latter two fragments comigrate under the gel conditions used. The left junction and central fragments contain 264 and 295 bp of IS*10* sequence respectively and even together give a much less intense hybridization signal than the right junction fragment, which contains 1025 bp of IS*10* material (Figure 3, lanes 2 and 4).

All ten revertants retain the light 2-kb band, con-

TABLE 1

Frequency and genetic analysis of Gal^R revertants

Strain	Gal^R frequency	Among 90 Gal^R revertants			
		Aro$^+$Pgl$^+$	Aro$^-$Pgl$^+$	Aro$^-$Pgl$^-$	% Aro$^-$Pgl$^{+/-}$
nadA$^+$	0.40×10^{-5} (≡1)	90	0	0	0/90 = <1%
nadA::IS*10-kan*	0.44×10^{-5} (1.1)	82	1	7	8/90 = 9%
nadA::IS*10-HH104-kan*	8.4×10^{-5} (23)	12	7	71	78/90 = 87%

Three isogenic strains were tested for the frequency of reversion to Gal^R as described in ROBERTS *et al.* (1985). Single Gal^R revertant colonies were picked and tested for AroG and Pgl phenotypes as described in MATERIALS AND METHODS. For each strain, ten colonies were picked from each of nine plates representing nine independent cultures. The three strains were NK7434 (*nadA*$^+$), NK7887 (*nadA*::IS*10-kan*) and NK7888 (*nadA*::IS*10-HH104-kan*).

TABLE 2

Effect of RecA$^-$ and/or IS*10* antisense RNA on frequency of Gal^R revertants

Strain	Frequency Gal^R ($\times 10^{-5}$)					
	RecA$^+$ at antisense RNA level:			RecA$^-$ at antisense level:		
	None	Low	High	None	Low	High
nadA$^+$	0.13	0.05	0.06	0.10	0.081	0.042
nadA::Tn*10*	0.67	0.05	0.03	1.5	0.17	0.046
nadA::IS*10-HH104-kan*	3.5	0.37	0.024	2.3	0.18	0.033

Three isogenic RecA$^+$ strains were transduced to *recA56* (MATERIALS AND METHODS), transformed with three multicopy plasmids that provided varying levels of IS*10* antisense RNA, and tested for the frequency of Gal^R revertants. The three strains were NK7434 (*nadA*$^+$), NK7435 (*nadA*::Tn*10*) and NK7888 (*nadA*::IS*10-HH104-kan*). The three plasmids were pNK1449 (which specifies a relatively low level antisense RNA typical of wild-type IS*10*), pNK1474 (which specifies a relatively high level of antisense RNA due to the *G8* mutation which increases the strength of the antisense RNA promoter) (SIMONS *et al.* 1983) and pNK1440 (which exerts no antisense control due to the presence of the *mci1* mutation that blocks antisense RNA pairing) (KITTLE *et al.* 1989).

TABLE 3

Gal^R and/or transposition frequencies for parental *nadA*::IS*10-HH104-kan* strains and selected Gal^R deletion derivatives

Strain	Frequency Gal^R	Total transposition frequency
nadA::IS*10-HH104-kan*		
#107	14×10^{-5}	5×10^{-3}
#109	18×10^{-5}	3.3×10^{-3}
Gal^R derivatives of #107		
#181		2×10^{-3}
#182		3.2×10^{-3}
#185		1.7×10^{-3}
#186		2.4×10^{-3}

Two independently constructed *nadA*::IS*10-HH104-kan recA56* strains, #107 and #109, and four independent Gal^R derivatives of #107 were tested for the frequency of transposition of the IS*10* element by a mating-out assay (MATERIALS AND METHODS). All of these strains have been analyzed physically in Figures 3 and 4. Parental strains #107 and #109 are in lanes 2 and 4 of Figure 3; #107 is in lanes 2 and 3 of Figure 4; revertants 181, 182, 185 and 186 are in lanes 4, 5, 8 and 9 of Figure 3 and lanes 4–11 of Figure 4. The "total transposition frequency" presented here is calculated as ten times the observed ratio of Kan^R exconjugants to total exconjugants, because control experiments have shown that in such strains approximately 10% of all transposition events occur into pOX38 and 90% into the bacterial chromosome (N. KLECKNER, unpublished results). For the two parental strains, the frequency of transposition events was compared directly to the frequency of Gal^R revertants: aliquots of the exponentially growing donor cultures were diluted and plated on Gal^R selective plates immediately prior to mixing those same cultures with recipient cells to assay for mating. For each strain assay, the number presented represents the average of ten independent cultures.

sistent with physical integrity of the IS*10* element at its *gal*-distal junction (see also below). Seven of the 10 Gal^R revertants lack the dark 3-kb band, and exhibit a single new dark band which varies in size from one isolate to another but is never less than 1025 bp, the distance from the *gal*-proximal *Eco*RV site to the end of IS*10* (Figure 3, lanes 5–14). This is the pattern expected for a series of adjacent deletions extending from the IS*10* element across *gal* and having endpoints at different positions in the adjacent target DNA.

Three of the ten revertants lack the right junction fragment but also exhibit more than a single new dark IS*10*-containing band. These clones probably contain an adjacent deletion plus a new IS*10* insertion at a different site. In the present experiments, such IS*10* transpositions might have occurred either before or after the adjacent deletion event. The existence of extra insertions in 30% of isolates is not surprising, because the *HH104* element is extremely active. As an example, during introduction of the *rec*A mutation into the RecA$^+$ *nadA*::IS*10-HH104-kan* strain, DNA was analyzed for four different P1 transductants which were isolated in four different single colony isolates of the recipient strain. Two of the four isolates contained a single IS*10* element (Figure 3, lanes 2 and 4); however, two of the isolates contained more than one IS*10* element (Figure 3, lanes 1 and 3), probably three or more in both cases, as judged by the number of darkly hybridizing bands. Multiple elements were observed despite the fact that each transductant had undergone only another 30 generations prior to DNA analysis. To avoid complications resulting from the occurrence of extra IS*10* insertions, the Gal^R revertants analyzed in lanes 5–14 were obtained from independent single clones obtained by plating out the exact culture whose DNA was analyzed in lane 2.

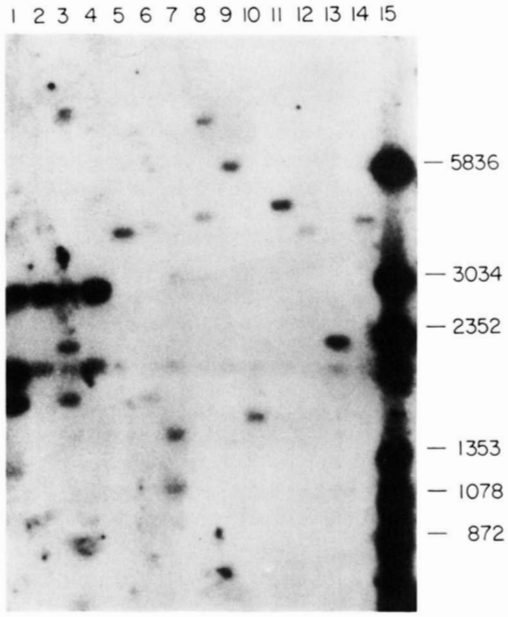

FIGURE 3.—Southern blot analysis of four independently constructed *recA56* parental strains carrying *nadA*::IS*10-HH104-kan* (lanes 1–4) and ten Gal^R revertants of one such strain (lanes 5–14). Parental strains are derived from NK7888 as described in Materials and Methods; the parent for all revertants was the parental strain in lane 2. DNA was digested with EcoRV and probed with IS*10* sequences (MATERIALS AND METHODS; Figure 2). Lanes 2 and 4 exhibit the bands expected for the parental strain; lanes 1 and 3 exhibit the expected bands plus additional bands indicative of the presence of multiple IS*10* insertions in these strains. Lane 15 contains size markers of 5386, 3034, 2352, 1353, 1078, and 872 bp.

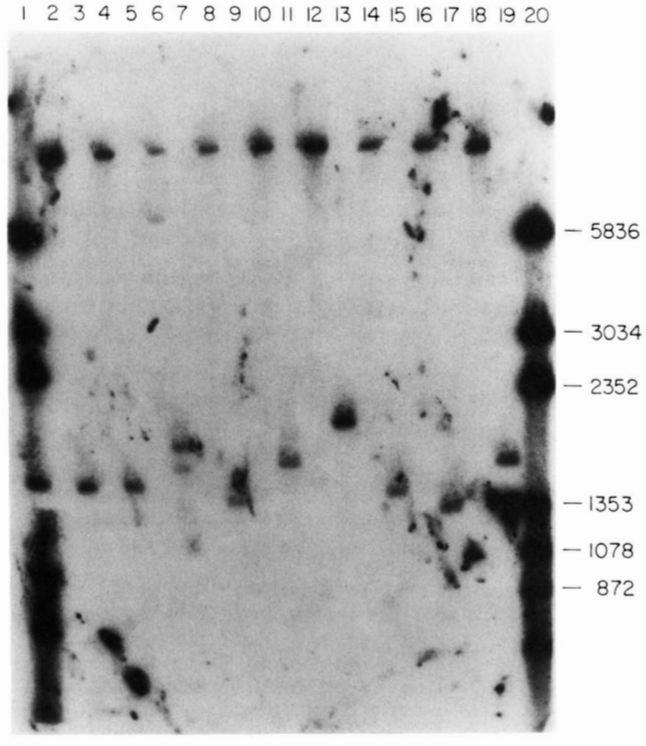

FIGURE 4.—Southern blot analysis of a parental *recA56* *nadA*::IS*10-HH104-kan* strain (lanes 2 and 3) and of its eight Gal^R revertants (lanes 4–19). The parental strain is that shown in lane 2 of Figure 3; the revertants are those in lanes 5, 6 and 9–13 in Figure 3. Lane 20 contains the same size markers as in Figure 3.

For 8 of the 10 Gal^R revertants analyzed, the location of the alteration at the IS*10*/*nadA* junction was examined at higher resolution by probing the corresponding DNAs after digestion with *Nde*I or *Sau*3a (Figures 2 and 4). *Nde*I cleaves within IS*10,* 60 bp from the right (*gal*-proximal) end of the parental IS*10* insertion and generates a single detectable IS*10*-containing fragment of about 8 kb. The presence of this fragment in all eight revertants demonstrates that the *gal*-distal parental *nadA*::IS*10-HH104-kan* junction, as well as all sequences within the transposon itself are present intact up to a point 60 bp from the *gal*-proximal IS*10*/*nadA* junction (Figure 2).

*Sau*3a cleavage gives a single detectable fragment of 1400 bp. This fragment corresponds to cleavage within IS*10,* 1320 bp from the right junction, and outside of IS*10* about 80 bp beyond the junction (Figure 2). Thus, a change in the size of this fragment in a Gal^R revertant whose *Nde*I fragment is intact indicates that the revertant in question has suffered a disruption in the galproximal IS*10*/*nadA* junction somewhere between bp 60 of IS*10* (the *Nde*I site) and 80 bp from the end of the element (the *Sau*3a site). This is the pattern expected for an IS*10*-promoted adjacent deletion. Seven of the eight Gal^R revertants lack the parental *Sau*3a fragment and exhibit a new fragment which is either somewhat larger or slightly smaller than the parental fragment (Figure 4). As expected for IS*10*-promoted deletions, none of the new junction fragments is smaller than 1320 bp, indicating that, to the resolution of this analysis, none of the deletions extends into the IS*10* element itself. The eighth revertant exhibits the same size *Sau*3a junction fragment as the parent. Since this revertant exhibits an altered *gal*-proximal junction fragment with *Eco*RV (above), and since *Sau*3a cleaves DNA frequently, about once every 256 bp, it seems likely that this revertant has suffered a deletion event in which the parental *Sau*3a junction fragment has been replaced with a new junction fragment which happens to be the same size as the parental fragment. *Sau*3a also cleaves within IS*10* close to the left junction and at the junction of IS*10* and *kan* sequences; this fragment was not detected in these blots.

Relative frequencies of IS*10* transposition and IS*10*-promoted adjacent deletions: For *nadA*::IS*10-HH104-kan,* the frequency of transposition is 20 to 30 times higher than the frequency of adjacent deletions, as shown by measurement of both frequencies in the same cultures of the appropriate strain (Table 3).

For wild-type *nadA*::IS*10-kan,* the frequency of transposition is also much higher than the frequency of adjacent deletions as judged by separate comparisons of the two events in strains carrying the wild-type

and *HH104* elements. The frequency of transposition for a *lacZ*::IS*10-kan* element is 10–40 times lower than the frequency of transposition of the isogenic *lacZ*::IS*10-HH104-kan* insertion (ROBERTS *et al.* 1985). At the *nadA* site, the frequency of adjacent deletions promoted by the two elements is at least 100-fold lower for the wild type element than for the *HH104* element. This estimate is obtained from the data in Table 1, with the assumption that approximately 10% of all Gal^R derivatives obtained in the wild-type case are the result of IS*10*-promoted adjacent deletions (see discussion of this point in Table 3, legend).

Relative frequencies of Tn*10* and IS*10*-promoted adjacent deletions: *nadA*::IS*10*-promoted adjacent deletions occur at about 10% the frequency of spontaneous Gal^R derivatives (Table 1; above). In contrast, *nadA*::Tn*10*-promoted adjacent deletions occur five to ten times more frequently than spontaneous Gal^R derivatives [Table 2; ROBERTS (1986) discussed above] . Thus, the frequency of Tn*10*-promoted adjacent deletions is 50 to 100 times greater than the frequency of IS*10*-promoted adjacent deletions. This difference implies that that Tn*10*-promoted events really are *Tn10*-promoted and not *IS10*-promoted.

DISCUSSION

The results presented above suggest that IS*10* does promote the formation of adjacent deletions which have the classical structure of adjacent deletions promoted by other IS elements but that the rate of formation of such deletions is much lower than either the rate of intermolecular IS*10* transposition or the rate of Tn*10*-promoted intrachromosomal rearrangements. The difference between the frequency of IS*10*-promoted adjacent deletions and the frequencies of these other events suggests that such deletions do not occur as the consequence of intramolecular but otherwise mechanistically normal transposition.

Elements which promote primarily replicative transposition (Mu and elements of the Tn*3*/gamma-delta family) readily promote adjacent deletions as the consequence of intra-molecular cointegrate formation (PATO 1989; SHERRATT 1988). Thus, the paucity of IS*10*-promoted deletions provides additional evidence that IS*10* does not use a cointegrate mechanism for transposition [see KLECKNER (1988) for further discussion of this issue].

How then do IS*10*-promoted adjacent deletions arise? We would like to suggest two models. In the first model, IS*10* could promote adjacent deletions by a transposition reaction that is normal in every way except that the two IS*10* ends provided by IS*10* elements present on two branches of a single replication fork interact with an adjacent target site. A particular version of this model is shown in Figure 5. In the example, the donor chromosome is only partially replicated, the target site selected is ahead of the replication fork, and the leading and lagging strands are assigned in a particular way. In this model, the "right" end of the IS*10* element on one sister (T) and the "left" end of the IS*10* element on the other sister (B) interact with an appropriately oriented target site by a standard "cut and paste" mechanism. After degradation of extraneous replicated chromosome arms, shown here to be the lagging strands of the replication forks since these strands may be more labile than the leading strands, this interaction generates two intact circles, each of which contains a copy of IS*10*. Together the two circles contain all the sequences of the original genome and each circle is an IS*10*-promoted deletion with respect to that genome. For IS*10*-promoted adjacent deletions in the bacterial chromosome, one of these circles would contain essentially the entire chromosome except for the small deleted region, and the second circle would contain the deleted sequences plus a second IS*10* element. When the target site is located within the already replicated region behind the fork(s), the same sequence of events also generates one circular adjacent deletion product and one linear fragment (not shown).

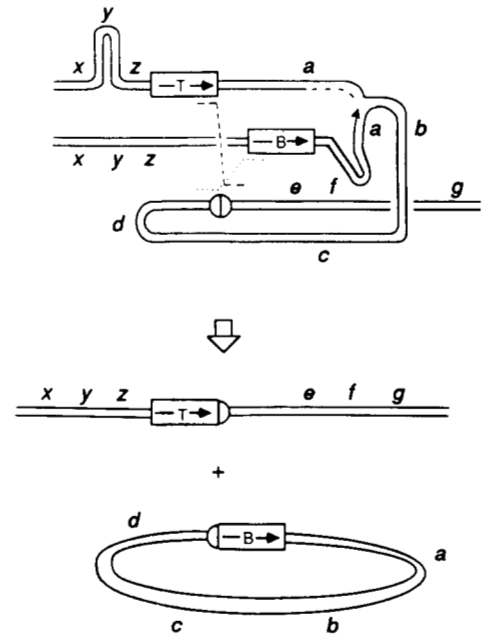

FIGURE 5.—Model for the formation of IS*10*-promoted adjacent deletions.

The final products of the proposed events are identical to those produced by intramolecular cointegrate formation. However, replication of the transposon has been provided in this case by replication of the donor chromosome prior to transposition rather than as an integral part of the transposition event itself. This model can also generate the two other classical products of replicative transposition: intermolecular cointegrates and intra-molecular replicative inversions. LI-

CHENS-PARK and SYVANEN (1988) have independently proposed a very similar model to account for rare formation of cointegrate molecules by IS*50*, an element which probably also transposes by a nonreplicative mechanism.

According to a second model, adjacent deletions might arise as abortive intramolecular transposition events in which one IS*10* end becomes separated from flanking donor sequences and joined to an adjacent target site without double strand excision at the other end of the element. Several variations on such an event can be imagined, depending upon the detailed nature of the transposition reaction itself. Since normal IS*10* transposition probably involves the strong and intimate interaction of transposase protein molecules bound at both ends of the element, it seems likely that even an apparently single-ended event would require participation of the other end of the element as a silent partner for formation of a synaptic complex. The silent end might undergo no covalent alteration, or might be cleaved on only one strand.

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