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Physical Analysis of Tn*10*- and IS*10*-Promoted Transpositions and Rearrangements

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ABSTRACT

We have investigated by Southern blot hybridization the rate of IS*10* transposition and other Tn*10*/IS*10*-promoted rearrangements in *Escherichia coli* and *Salmonella* strains bearing single chromosomal insertions of Tn*10* or a related Tn*10* derivative. We present evidence for three primary conclusions. First, the rate of IS*10* transposition is approximately 10^{-4} per cell per bacterial generation when overnight cultures are grown and plated on minimal media and is at least ten times more frequent than any other Tn*10*/IS*10*-promoted DNA alteration. Second, all of the chromosomal rearrangements observed can be accounted for by two previously characterized Tn*10*-promoted rearrangements: deletion/inversions and deletions. Together these rearrangements occur at about 10% the rate of IS*10* transposition. Third, the data suggest that intramolecular Tn*10*-promoted rearrangements preferentially use nearby target sites, while the target sites for IS*10* transposition events are scattered randomly around the chromosome.

Tn*10* is a "composite" transposable element; its ends are inverted repeats of insertion sequence IS*10* (Fig. 1). IS*10*-Right encodes as essential transposase protein that acts at the ends of Tn*10* to promote transposition of the whole element and at the two ends of a single IS*10* to promote transposition of an individual insertion sequence. The termini of IS*10*-Left are intact but IS*10*-Left does not encode a functional transposase protein (FOSTER et al., 1981a; WAY and KLECKNER 1984; ROBERTS *et al.* 1985). Existing evidence strongly suggests that Tn*10* transposes by a nonreplicative transposition mechanism in which transposon sequences are excised from the donor site and inserted into a target site without extensive replication (BENDER and KLECKNER 1986; MORISATO and KLECKNER 1984; WEINERT *et al.* 1984).

Throughout the work to be described below, the following conventions will be observed with regard to nomenclature.

1. The two ends of an individual IS*10* element are not genetically identical, and are referred to as "inside" and "outside" ends respectively according to their locations in wild type Tn*10*. Tn*10* transposition involves the action of transposase at two "outside" ends; IS*10* transposition involves one "outside" and one "inside" end; certain other transposon-promoted events (below) involve the two "inside" ends.

2. Some of the experiments described below involve strains containing wild type Tn*10*; others involve an element that we call Tn*Gal* which consists of direct repeats of IS*10* (one IS*10*-Left and one IS*10*-Right) flanking the *E. coli gal* operon (Figure 1; *nadA*::Tn*Gal* was isolated as a Tn*10*-promoted rearrangement of a *nadA*::Tn*10* rearrangement). Because of the different structures of these two elements, it is necessary to make a distinction between "inside" IS*10* ends and "internal" IS*10* ends. In the case of Tn*10*, the IS*10* ends which are "internal" to the composite element, adjacent to the central nonrepeated material, are also by definition genetically two "inside" ends. However, in the case of Tn*Gal*, one "internal" IS*10* end is genetically an "inside" end but the other one is genetically an "outside" end.

3. One issue of interest is whether a single IS*10* element can promote recombination events other than transposition. In a strain that originally contains either Tn*10* or Tn*Gal*, we will use the term "Tn*10*-promoted events" to refer to events in which both IS*10*-Left and IS*10*-Right are thought to participate, and the term "IS*10*-promoted events" to refer to events in which only one of the two IS*10* sequences is thought to participate.

Genetic and physical analysis has revealed several types of Tn*10*-promoted recombination events other than transposition. The most prominent of these events involve the interaction of internal ends and a target site. The first such events to be described were two alterations of chromosomal material adjacent to an inserted Tn*10* element: Tn*10*-promoted deletions and Tn*10*-promoted deletion/inversions. Both events are most easily explained as the *intra*molecular attack of the two inside IS*10* ends on a nearby target site.

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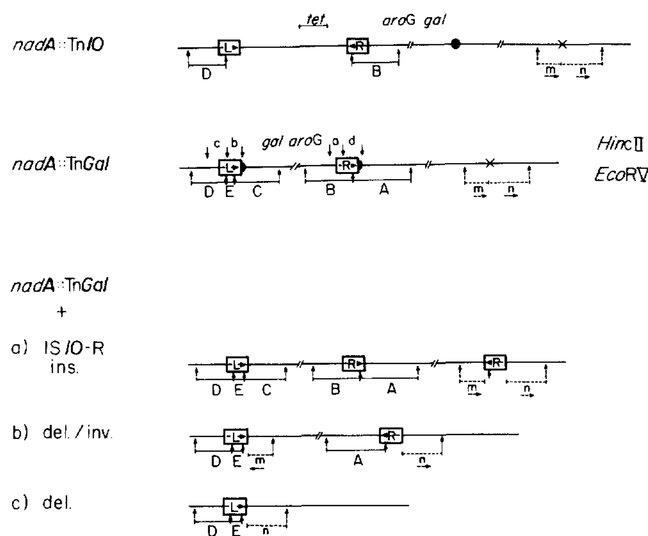

FIGURE 1.—(*Top*) Structures of *nadA*::Tn*10* and *nadA*::Tn*Gal*. *nadA*::Tn*Gal* was obtained as a Tet^S deletion/inversion derivative (Figure 2) of *nadA*::Tn*10* to the target site indicated by a filled circle (RALEIGH and KLECKNER 1984). *Hinc*II and *Eco*RV junction fragments of the IS*10* elements in *nadA*::Tn*Gal* are shown; *Eco*RV fragments B and D are the same as the original outer junction fragments of *nad*A::Tn*10*. The sizes of *Eco*RV fragments A–E are 3600, 2900, 2650, 2250 and 657 bp, respectively; the sizes of *Hinc*II fragments a–d are 780, 1000, 1100 and 1175, respectively. The distance between the IS*10* elements in Tn*Gal* is not known exactly, but is greater than 6 kb. (*Bottom*) Structures of three common transposon promoted rearrangements of *nad*A::Tn*Gal*. In each case, target site "×" is used, and the locations of adjacent segments "m" and "n" in each rearrangement are indicated. The IS*10* insertions, deletion/inversion and deletion correspond to Classes 1, 5 and 4 respectively in Table 3.

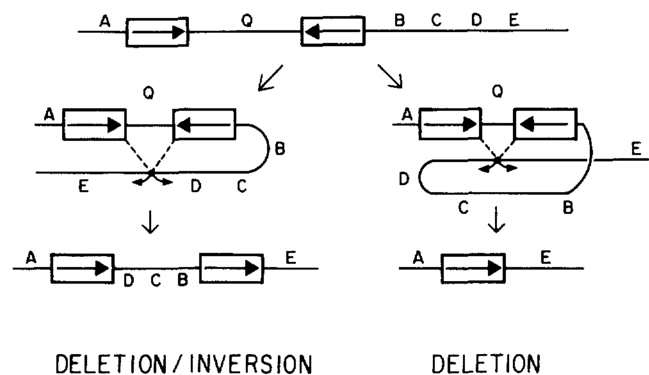

FIGURE 2.—Structures and proposed model for formation of Tn*10*-promoted deletion/inversions and Tn*10*-promoted deletions. Note that in each case two internal IS*10* ends interact with the target and the internal nonrepeated portion of the original transposon is lost.

As shown in Figure 2, both events result in deletion of the nonrepeated Tn*10* material located between the two IS*10* elements and, depending upon the orientation of the target with respect to the element, either deletion or inversion of a contiguous DNA segment extending from the adjacent target site to the nearest inside IS*10* end. The *inter*molecular equivalent of these rearrangements, in which the two inside ends interact with a target site on another replicon, has also been observed; this event is variously referred to as "inside-out" or "inverse" transposition (FOSTER *et al.* 1981a; CHANDLER *et al.* 1979; HARAYAMA, OGUCHI and INO 1984b).

Some transposable element make structures called cointegrates. These structures arise by a replicative process that results in duplication of the transposable element itself. The available evidence suggests that neither the full Tn*10* element nor an individual IS*10* element can generate cointegrates (BENDER and KLECKNER 1986; WEINERT *et al.* 1984; HARAYAMA, OGUCHI and INO 1984a).

The above picture of Tn*10*-promoted events is the aggregate of a number of different experimental approaches. In general, one or more events has first been selected genetically and the nature of the events answering the selection then determined by genetic or physical analysis. For example, Tn*10*-promoted deletions and deletion/inversions were identified as tetracycline-sensitive derivatives of a Tn*10* insertion in the Salmonella histidine operon (KLECKNER, REICHARDT and BOTSTEIN 1979). The structures of large numbers of derivatives were deduced genetically; physical confirmation of the structure was obtained by analysis of a small number of similar derivatives isolated from a Tn*10* insertion in bacteriophage λ (ROSS, SWAN and KLECKNER 1979).

In the experiments described below, Southern blot hybridization analysis was used to confirm and extend the above picture in the following four ways.

First, we wished to determine the frequency of transposition of an individual IS*10* element. To do so, we screened by Southern blot hybridization a large number of unselected clones of IS*10*-containing strains to identify those in which a transposition (or other rearrangement of IS*10* sequences) had occurred. Previously IS*10* transposition frequencies could only be estimated using an artificial 'marked IS*10*' element containing an inserted selectable marker. This approach also addresses directly the issue of whether any other IS*10*- or Tn*10*-promoted recombination event(s) might occur at a frequency comparable to IS*10* transposition.

Second, we wished to confirm by physical analysis of a large number of derivatives a previous conclusion from genetic analysis, that the vast majority of rearrangements resulting in loss of a marker located between two IS*10* sequences events result from the two Tn*10*-promoted rearrangements, deletions and deletion/inversions, and as above to determine whether some other event might be present as a minority class. Southern blot analysis was applied to derivatives of Tn*10* and Tn*Gal* selected for loss of tetracycline resistance or galactose genes respectively.

Third, we wished to examine physically a set of Tn*10* derivatives that had undergone transposon-promoted rearrangements to target sites within the ele-

ment itself to see whether the spectrum of observed rearrangements was different for this special, limited target region than for rearrangements in general. We have therefore examined a particular subset of Tet^S derivatives of a Salmonella *hisG*::Tn*10* insertion, those which retained the ability to revert to His^+ and thus had undergone rearrangements of sequences wholly internal to the element.

Fourth, we wished to examine further, by physical analysis of a larger number of derivatives, our previous conclusion that a cell containing one genetically selected IS*10*- or Tn*10*-promoted event has an increased probability of containing a second (unselected) transposon-promoted event, as if the first and second events are not independent (RALEIGH and KLECKNER 1984).

The primary conclusions of this work are: (1) The rate of IS*10* transposition is approximately 10^{-4} per element per cell generation in minimal medium. (2) The second most frequent type detected was Tn*10*-promoted alteration of adjacent sequences (deletions and deletion/inversions). The rates of these two events together are approximately 1×10^{-5} per element per generation, about 10% the rate of IS*10* transposition. At least 90% of all events resulting in loss of a marker between two IS*10* sequences have the physical structures expected for events of this type. The physical structures of the remaining 10% of events were complex or/and uninterpretable. (3) All 33 of the internal (His-revertible) Tet^S transposon-promoted rearrangements analyzed could be explained as Tn*10*-promoted deletions or deletion/inversions to target sites within the element itself. Twenty-one of the derivatives probably used as a target the 9 bp repeat sequence at the ends of Tn*10*, which is known to be a favored target site for all types of Tn*10*-promoted events (references below). IS*10*-promoted adjacent deletions, which have been proposed to occur in other situations (WEINERT *et al.* 1984), were not detected; if they occur, their frequency must be less than 10% that of Tn*10*-promoted deletion/inversions, and their rate less than about 10^{-6} per element per generation. (4) Some aspects of the data raise the possibility that intramolecular events preferentially use nearby target sites, while the target sites for transposition events are scattered randomly around the chromosome. (5) The data obtained were consistent with previous observations suggesting the occurrence of "multiple" transposon-promoted events.

MATERIALS AND METHODS

Media and enzymes: LB and M9 minimal media and agar have been described (MILLER 1972). Minimal media included 0.2% (w/v) glycerol as a carbon source, 1 ng/ml nicotinamide, and, where appropriate, 1.0% (w/v) galactose. MacConkey indicator agar (Difco) was supplemented with 1.0% (w/v) sorbitol. Antibiotics (Sigma) were routinely used at 100 μg/ml (ampicillin) or 20 μg/ml (chloramphenicol and tetracycline). Plates for the selection of tetracycline-sensitive clones ("Tet^S plates") are described by BOCHNER *et al.* (1980) as modified by MALOY and NUNN (1981). Phenol red plates are described by GEORGE and LEVY (1983).

Restriction enzymes, T4 DNA ligase, DNA polymerase I, and OX174 DNA were purchased from New England Biolabs. Procedures for restriction enzyme digestions and ligations were as described (MANIATIS, FRITSCH, and SAMBROOK 1982).

Bacterial strains: NK7176 is W3110 *nadA*::Tn*10*; NK7381 is a spontaneous Tet^S derivative of NK7176; NK7383 is a *recA*$^-$ *srl*$^+$ derivative of NK7381 made by first transducing NK7381 to *recA srl*::Tn*10* using NK5841 as a donor strain, and subsequently reverting the transductant to *srl*$^+$. NK5841 is Hfr KL16 *ilv thr spc recA56 srl*::Tn*10*. The transposons in NK7176 and NK7381 are described in RALEIGH and KLECKNER (1984) and Figure 1.

Plasmids: pNK997 is derived from the IS*10*-containing plasmid pNK82 (FOSTER *et al.* 1981a) in two steps: insertion of a *plac*UV5 promoter at the *Bcl*I site (bp 70) of ISD*10*-Right to create pNK353 (MORISATO *et al.* 1983) and elimination of the "outside" end of IS*10*-Right bp1-70 by a deletion between an *Eco*RI site in the *plac*UV5 fragment upstream of the promoter and an *Eco*RI site in upstream vector sequences. pNK997 overproduces transposase at approximately 100 times the level of pNK82 but cannot itself give rise to transpositions of IS*10*. When rearrangements were obtained in strains carrying pNK997, this plasmid was eliminated from the rearranged derivative prior to Southern blot analysis by transformation with and selection for an incompatible Cam^R Amp^S plasmid, pNK259. Cam^R transformants were screened for sensitivity to ampicillin on phenol red plates. pNK259 is pBR323 containing an insertion at its *Pst*I site of the *Pst*I *camR* fragment of Tn9 (J. WAY and N. KLECKNER, unpublished data).

Southern hybridization: procedure and analysis: Extraction of chromosomal DNA and Southern blot hybridization were performed exactly as described by RALEIGH and KLECKNER (1984). Probes used were either the *Sau*3A fragment of IS*10*-L (base pairs 67–1320) from pNK83, or the complete plasmid pNK290 (FOSTER *et al.* 1981a). The *Sau*3A fragment was purified by electroelution from a 4% polyacrylamide gel.

Rates of transposition and rearrangement: The rate of events per element per generation (R) is calculated from the frequency of events observed in an experimental population (f) by the equation $R = 0.4343f/(\log N - \log N_0)$ from DRAKE (1970). N is the total number of cells in the population at the time of plating. N_0 is the number of cells in the population at the beginning of the experiment. For these experiments, $N_0 = 1$ because all cultures were grown from single colonies which in turn arose from single cells, and $N = 5 \times 10^9$.

Experimental protocols: The data in Tables 2 and 4 were obtained by protocols summarized in Table 1 and below. Five separate experiments (1–5) were performed, each of which began with one single colony of the relevant bacterial strain lacking any plasmid (Table 1, step A). In each experiment this single colony was restreaked onto one or more different non-selective plates (step B); each such restreak is denoted by a different lower case letter (a, b, etc.). For experiment 1, the "restreaking" in step B was replaced by transformation of a small culture grown from the starter colony; the culture was either transformed with pNK997 and plated on ampicillin-containing plates (c, d) or mock-transformed with no plasmid and plated on non-ampicillin-containing plates (a, b). From the "restreaking"

TABLE 1

Summary of experimental protocols

Step	Details	Experiment													
		1				2		3	4			5			
A	Strain (one primary colony per experiment):	NK7383				NK7383		NK7381	NK7176			NK7176			
B	Sub-experiment:	1a	1b	1c	1d	2a	2b	3	4a	4b	4c	5a	5b	5c	5d
	Primary colony restreaked on:	MG	MG	MGA	MGA	MG	MG	LB	LB	LB	LB	MG	LB	LB	LB
C	No. of colonies chosen from (B):	72	72	72	72	32	33	33	35	72	72	72	72	72	72
D	Colonies from (C) plated either selectively on:		MGG		MGGA	MGG	MGG		TS						
	or nonselectively on:	MG		MGA				MG		TS-CT	TS-FA	MG	LB	LB	LB
E	Purification of colonies from (D)														
	No. purifications:	2	2	2	2	0	0	1	2	2	2	0	0	2	2
	Medium														
	1st:	MG	MG	MG	MG			MG	TS	TS-CT	TS-FA			LB	TS-CT-FA
	2nd:	MG	MG	MG	MG				LB	LB	LB			LB	LB

MG, MGA, MGG and MGGA are M9 media containing glycerol, glycerol and ampicillin, glycerol and glucose, or glycerol, glucose and ampicillin, respectively. LB is tryptone plus yeast extract, a rich medium. TS are plates selective for tetracycline-sensitive cells (also called Tet^S plates). TS-CT, TS-FA and TS-CT-FA are TS plates lacking one or two of the ingredients necessary for the selection, chlortetracycline (CT) or/and fusaric acid (FA).

plates, a large number of different colonies (each being an independent clone of cells derived from a single cell), were chosen (step C), grown slightly, and replated on medium selective either for Tet^S or Gal^R derivatives or on medium that did not select for any transposon-promoted rearrangement (step E). One Gal^R or Tet^S colony or, alternatively two or three colonies not subjected to selection, were chosen from each independent clone. Each such chosen colony was either analyzed directly without purification or purified according to one of several regimes (step E). For experiment 1, "purification" actually involved transformation by pNK259 (to eliminate pNK997) followed by a single restreaking.

We are confident that all of the rearrangements identified occurred during growth of the many independent clones. Only occasionally did derivatives from two different clones exhibit the same pattern of IS*10*-containing restriction fragments, and there are several possible explanations for such coincidences. Furthermore, in no case did the particular purification regime have any discernible effect on the frequency or types of rearrangements observed, even in experiments where different regimes were compared directly.

RESULTS

IS*10* transposition in unselected clones: Many independent clones of strains containing either *nadA*::Tn*10* or *nadA:TnGal* (Figure 1) were plated for single colonies. DNA was extracted from a large number of such colonies after amplification by a few additional generations of growth in liquid culture. In most cases, several independent clones were pooled prior to the amplification and extraction steps. The extracted DNA was digested with restriction enzyme *Eco*RV, fragments were separated by agarose gel electrophoresis, and IS*10*-containing fragments were identified by Southern blot hybridization with a radioactive probe containing IS*10* sequences. For any pool that yielded an IS*10*-containing fragment other than one of those present in the parental strain, each clone within that pool was individually analyzed further to determine the nature of the identified rearrangement. Additional details are provided in MATERIALS AND METHODS.

A clone was said to have undergone a simple transposition of IS*10* if it retained all four parental IS*10* "junction fragments" (those fragments containing the junctions between IS*10* sequences and adjacent chromosomal or unique transposon sequences) and also gave rise to two new IS*10*-containing fragments (see Figure 1, bottom). Transpositions of IS*10*-Right and of IS*10*-Left are distinguished by *Eco*RV digestion. IS*10*-Right contains a single asymmetrically-located *Eco*RV site, and IS*10*-Right insertions therefore yield one "dark" and one "light" junction fragment due to the differential hybridization of the IS*10* probe to the different amounts of IS*10* sequence present in each fragment; IS*10*-Left contains two *Eco*RV sites located roughly symmetrically near the ends of the element, and IS*10*-Left insertions correspondingly yield two "light" junction fragments.

These criteria for IS*10* transposition are subject to two assumptions. First, transposition of a full Tn*10* or Tn*Gal* element would have given the same fragment pattern as transposition of IS*10*-Left or of IS*10*-Right respectively; we assume that none of the observed events are transpositions of the complete composite

TABLE 2

IS10 transposition in unselected clones

Expt.	Strain	Genotype	Medium	Total clones analyzed	IS*10* insertions observed R/L/Ambig = Total	IS*10* transposition Frequency	IS*10* transposition Rate
1a	NK7383	*nadA*::Tn*Gal recA*$^-$	Minimal	180 } 252	1/0/0 = 1 }	2/252 = 8×10^{-3}	4×10^{-4}
5a	NK7176	*nadA*::Tn*10 recA*$^+$	Minimal	72	0/1/0 = 1 }		
1c	NK7383	*nadA*::Tn*Gal recA*$^-$/pNK997	Minimal	123	5/2/0 = 7 }	$\frac{7/123}{20} = 3 \times 10^{-3}$	1×10^{-4}
4b	NK7176	*nadA*::Tn*10 recA*$^+$	LB	72	5/1/1 = 7	$\frac{29}{324} = 9 \times 10^{-2}$	4×10^{-3}
4c	NK7176	*nadA*::Tn*10 recA*$^+$	LB	72	4/0/2 = 6		
5b	NK7176	*nadA*::Tn*10 recA*$^+$	LB	72	3/0/1 = 4		
5c	NK7176	*nadA*::Tn*10 recA*$^+$	LB	72	4/0/0 = 4		
5d	NK7176	*nadA*::Tn*10 recA*$^+$	LB	72	6/0/2 = 8		
				324	29		

pNK997 is *ptac*-transposase overproducer plasmid that increases 20-fold the frequencies of Tn*10* and Tn*Gal* transposition, Tn*Gal*-promoted rearrangements (Gal^R) and IS*10* transpositions present as secondary events in such Gal^R rearrangements (data not shown). The frequency of IS*10* transposition in the presence of pNK997 is therefore assumed to be 20 times the frequency of transposition in the absence of the plasmid. "Ambig" means that the IS*10* insertion could not be classified as either IS*10*-R or IS*10*-L. Transposition rate is per element per cell generation (see MATERIALS AND METHODS).

element, because the frequencies of *nadA*::Tn*10* and *nadA*::Tn*Gal* transposition are less than 1% the frequencies of events observed here (M. M. SHEN, E. A. RALEIGH, D. ROBERTS and N. KLECKNER, unpublished). Second, in most cases this analysis cannot distinguish IS*10* transpositions from one particular type of replicative IS-promoted DNA rearrangement, "replicative inversion" or "insertion-inversion" [see for example SHAPIRO (1979) and KLECKNER (1981)]. The structure produced by this event is identical to that of an IS*10* insertion except that the chromosomal material located between the element present at the original location and the element at the new location has been inverted. We assume that none of the events we observe are replicative inversions because there is no evidence for high-frequency replicative IS*10*-promoted recombination: IS*10* does not make cointegrates at any detectable frequency (above); our analysis below and the analysis of WEINERT *et al.* (1984) have failed to reveal a high frequency of adjacent deletion events; also WEINERT *et al.* report that IS*10*-promoted replicative inversions did not occur at a detectable frequency in their plasmid transposition system.

nadA::Tn*10* and *nadA*::Tn*Gal* strains were examined for IS*10* transposition under a number of slightly different experimental regimes (Table 1 and MATERIALS AND METHODS). As summarized in Table 2, the aggregate of all experiments suggests that the rate of IS*10* transposition is 1–4 $\times 10^{-4}$ per element per cell generation in minimal medium, that the rate of transposition is ten times higher in rich medium (but see DISCUSSION), and that most of the observed transposition events are of IS*10*-Right.

Only one clone exhibited a pattern other than that of an IS*10* insertion: one deletion/inversion event was detected (in experiment 1c) as compared with a total of 38 IS*10* transposition events observed in all experiments. Conservatively we can conclude that rearrangements other than IS*10* transposition occur at less than 10% the rate of IS*10* transposition; the observed ratio, 1/38, corresponds to a relative rate of 3%. This analysis would not have detected any IS*10*- or Tn*10*-promoted event that resulted in complete excision of the element, because independent clones were first examined in pools rather than individually. Previous experiments suggest that complete excisions of Tn*10* occur at a rate of less than 10^{-6} per element per generation (KLECKNER, REICHARDT and BOTSTEIN 1979); the rate of loss of an individual IS*10* element is presently under investigation.

Physical analysis of derivatives selected for loss of a central transposon marker: Positive genetic selections exist both for loss of tetracycline-resistance from a strain carrying Tn*10* and for loss of galactose genes from a GalE$^-$ strain carrying Tn*Gal* (MATERIALS AND METHODS); the resulting derivatives are either tetracycline-sensitive (Tet^S) or galactose-resistant (Gal^R), respectively. 168 independent Gal^R derivatives of strains carrying *nadA*::Tn*Gal* and 35 independent Tet^S derivatives of a strain carrying *nadA*::Tn*10* were analyzed by Southern blot hybridization using radioactively labeled IS*10* sequences as a probe. Examples of the restriction fragment patterns generated by some Gal^R rearrangements are shown in Figure 1. The criteria used for interpretation of hybridization patterns for the Gal^R derivatives are summarized in Table 3, and representative Southern blot data are shown in Figure 3. Analogous criteria were used for analysis of Tet^S derivatives (not shown). The results of these experiments, described in Table 4, can be summarized as follows:

TABLE 3

Interpretation of Southern blot patterns of Gal^R derivatives

Description						Interpretation	
Type	Class	Enzyme	Total no. of bands	Parental bands remaining	Properties of new bands	Type of event	Location of target site
IS*10* insertion	1	RV	7	A, B, C, D, E	1 dk, 1 lt	Ins. of IS*10*-R	Between or outside of IS*10*s
	2	RV	7	A, B, C, D, E	2 dk	Ins. of IS*10*-L	Between or outside of IS*10*s
Deletions and deletion/inversions	3	RV	2	A	1 dk	Del	Leftward, beyond fragment E
	4	RV	3	D, E	1 lt	Del	Rightward, beyond fragment B
	5	RV	5	A, D, E	1 dk, 1 lt	Del/Inv	Leftward or rightward, beyond Fragments D or A
	6	RV	4	D, E	1 very dk, 1 lt sum is 4900 bp	Del/Inv	Fragment A
	7	RV	4	A, E	1 dk, 1 lt sum is 3550 bp	Del/Inv	Fragment D
	8	RV	4	A, D	2 dk, sum is 1990	Del/Inv	Fragment E
	9	RV	4	A, D, E	1 dk (same size; = length of IS10)	Del/Inv	IS*10*-R or *L*, but not in fragments A, D, E, c or d
		HII	3	c, d	1 dk (same size; = length of IS10)		
	10	RV	4	A, D, E	1 dk (same size; less than length of IS*10*)	Del	IS*10*-R or L, but not in fragments A, D, E, C or d
	11	RV	4	A, D, E	1 dk	Del/Inv	IS*10*-R, between *Hinc*II site at bp 393 and *Eco*RV site at bp 1062
		HII	3	c	2 dk		
Non-Tn*10*-promoted events	12	RV	0	None	None	Spontaneous deletion of entire TnGal element.	
		HII	0	None	None		
	13	RV	5	A, B, C, D, E	None	Spontaneous mutation in *galT* or *E*	
		HII	4	a, b, c, d	None		
	14	RV	3	A, D, E	None	Deletion by reciprocal recombination between IS*10*'s.	
		HII	2	c, d	None		

dk = dark; lt = light; RV = *Eco*RV; HII = *Hinc*II; restriction fragments identified in Figure 1.

1. Of the 168 Gal^R derivatives analyzed, 162 (96%) appear to be the result of Tn*Gal*- or IS*10*-promoted recombination events in that they contain one or more new IS*10*-containing *Eco*RV restriction fragments. Similarly, 34 of 35 (97%) of Tet^S derivatives were transposon-promoted.

2. The average frequency of Gal^R derivatives in all experiments was about 3×10^{-4}, which corresponds to a rate of occurrence per cell per generation of about 1.5×10^{-5}. This is essentially the same as the rate of 2.5×10^{-5} for Gal^R derivatives of Tn*Gal* previously determined by fluctuation analysis (RALEIGH and KLECKNER, 1984). The overall frequency of Tet^S derivatives of Tn*10* was the same as for Gal^R derivatives of Tn*Gal* and the same as previously observed for other Tn*10* insertions (KLECKNER, REICHARDT and BOSTEIN 1979).

3. Of the 162 transposon-promoted Gal^R derivatives, 149 (92%) can be accounted for as the products of Tn*Gal*-promoted deletions or deletion/inversions analogous to those previously described. Of the remainder, six derivatives (4%) contain complex events in which more than two new IS*10*-containing junction fragments appeared and which must therefore have involved more than a single transposon-promoted recombination event of any traditional type. One such derivative was an IS*10* insertion into the *gal* genes, and five such derivatives gave complex patterns that could not be definitively interpreted without more extensive analysis. An additional seven derivatives (4%) contained uninterpretable non-"multiple" (containing one or two new IS*10* junction fragments) events. None of the derivatives analyzed gave the pattern of bands expected for an IS*10*-promoted adjacent deletion from one "internal" IS*10* end into the *gal* genes.

4. The five derivatives that did not result from a transposon-promoted event include: two that show no change in IS*10*-containing fragments and are presumably point mutations in *galK* or *galT*, one that lacks all IS*10* sequences and is presumably (though not necessarily) a spontaneous deletion of the entire region, and two that have the structure expected for homologous recombination between the directly repeated IS*10* sequences of Tn*Gal*.

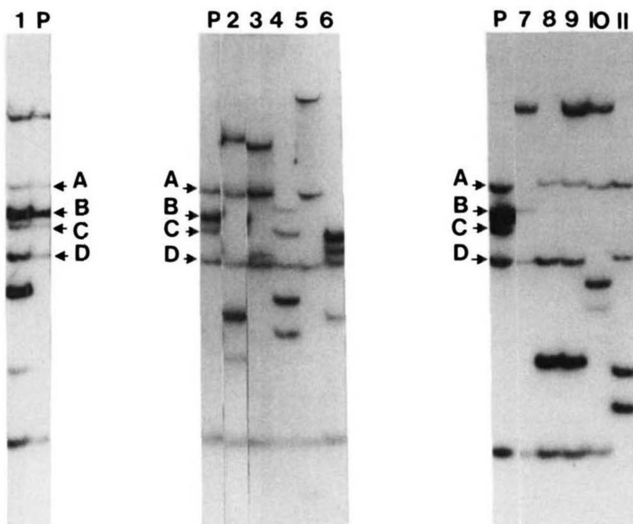

FIGURE 3.—Southern blot analysis of IS*10* rearrangements derived from *nadA*::Tn*Gal*. DNA was digested with *Eco*RV and probed with radioactive IS*10* sequences. Lanes marked "P" show bands A–E of the parental insertion (Figure 1). The rearrangements illustrated and their class in Table 3 are: lane 1, parent plus insertion of IS*10*-Right, Class 1; lanes 3 and 5, deletion/inversions, Class 5; lane 2, deletion/inversion of Class 5 plus insertion of IS*10*-Right; lane 4, deletion/inversion of Class 6 plus insertion of IS*10*-Right; lane 7, deletion rightward of Class 4; lanes 8 and 9, two independently isolated identical deletions ending in an IS*10* element, Class 10; lane 10, deletion/inversion leftward of Class 7; lane 11, deletion/inversion into IS*10*-Left, Class 8. The top band in lanes 1 and "P" of the first panel and lanes 7, 9 and 10 is from hybridization to contaminating plasmid DNA and should be disregarded. Size standards (not shown) were *Pst*I, *Acc*I and *Hae*III digests of OX174.

From these observations we conclude that Tn-promoted deletions and deletion/inversions are indeed the predominant transposon-promoted rearrangement obtained from this type of genetic selection, and find no evidence that any other type of simple event occurs at comparable frequency.

These observations are consistent with previous data suggesting that Tn*10* gives rise to "multiple" events at frequencies higher than expected for two independent events. In minimal medium, the frequency of multiple events among Gal^R derivatives (6/162 = 4%) is significantly higher than the frequency of any single event. In LB medium, the measured proportion of Tet^S deletion/inversion derivatives containing in addition a new IS*10* insertion (6/35 = 0.17) may be slightly higher than proportion of unselected clones containing a new IS*10* insertion (29/324 = 0.09). We suggest that the unusually high frequency of IS*10* transposition observed in rich medium in these experiments as compared with previous experiments has provided an exceptionally high background over which the occurrence of multiple events cannot be seen.

Deletions and deletion/inversions do not occur equally frequently. This is not an unusual result; in some experiments deletions are more frequent, and in others deletion/inversions are more frequent (KLECKNER *et al.* 1979; RALEIGH and KLECKNER 1984; WEINERT *et al.* 1984). These differences probably reflect at least in part the particular disposition of essential genes adjacent to the transposon; for example, the *nadA* locus is flanked by loci that are important for cell viability under the conditions used for selection of tetracycline-sensitive derivatives (E. A. RALEIGH, unpublished data). Such differences could also partly reflect intrinsic mechanistic features of the transposition process.

Physical analysis of revertible Tet^S derivatives of hisG9424::Tn*10*: Previous genetic analysis of Tet^S derivatives of a Salmonella *hisG*::Tn*10* insertion revealed about 20% which retained the ability to give His^+ revertants (KLECKNER, REICHARDT and BOTSTEIN 1979). Such derivatives should have suffered changes only within the Tn*10* element itself, since alterations outside of the element would have deleted or rearranged *hisG* sequences, thus precluding subsequent restoration of an intact *hisG* gene.

Among revertible derivatives, several types of events might have been expected: Tn*10*-promoted deletions and deletion/inversions to sites within either of the IS*10* sequences (Figure 4, lines b–e), insertions of an IS*10* element into the *tet* genes, or IS*10*-promoted adjacent deletions extending from one inside IS*10* end into the *tet* genes (Figure 4, line f). Rearrangements of the latter type are known to be promoted by the types of transposable elements that promote intermolecular cointegrate formation as the intramolecular equivalent of that event (for example, WEINERT *et al.* 1984). It has been suggested that IS*10* may also promote such deletions at low frequency (WEINERT *et al.* 1984); whether and how a non-replicative element might promote deletions of this type is an interesting question.

The potential target sizes for all of these events are very similar: 2658 bp for Tn*10*-promoted deletions and deletion/inversions into one of the two IS*10* sequences, about 2 kb for IS*10* insertions into the *tet* genes, 2.3 kb for adjacent deletions beginning at the inside end of IS*10*-Left and extending into the *tet* genes, 5.4 kb for adjacent deletions beginning at the inside end of IS*10*-Right and extending into the *tet* genes.

The results of this analysis are summarized in Table 5. The striking result is that all 33 of the derivatives resulted from a Tn*10*-promoted deletion/inversion or deletion into a target site within one of the IS*10* sequences. Furthermore, 21/33 of the events analyzed had apparently (given the resolution of the mapping performed) used as a target site one of the 9 bp repeat sequence present at either end of the original Tn*10* element. This preference is probably due to the fact that this particular sequence is known to be a favored site for Tn*10* insertion and for Tn*10*-promoted rear-

TABLE 4

Classes of Gal^R and Tet^S derivatives

Expt.	NK strain = genotype	Selection	Frequency Gal^R or Tet^S × 10^{-4}	Total	Non-IS*10* promoted	IS*10*/Tn-promoted								
							Single events[a]				Multiple Events[b]		
						Total	D/I	D	IS*10*	Un-cat	D/I + IS*10*	IS*10* + IS*10*	Un-cat
1b	7383 = *nadA*::Tn*Gal recA*⁻	Gal^R	1.4	70	0	70	49	18	0	2	0	1	0
2a	7383 = *nadA*::Tn*Gal recA*⁻	Gal^R	6	33	0	33	23	6	0	2	0	0	2
2b	7383 = *nadA*::Tn*Gal recA*⁻	Gal^R	2	32	1[c]	31	21	6	1	3	0	0	0
3	7381 = *nadA*::Tn*Gal recA*⁺	Gal^R	3	33	4[d]	29	19	7	0	0	0	0	3
				168	5	162	112	37	1	7	0	1	5
							149						
4a	7176 = *nadA*::Tn*10 recA*⁺	Tet^S	5	35	1	34	28	0	0	0	6	0	0

[a] One or two new IS*10*-containing junction fragments. D = deletion, I = insertion, Uncat = uncategorized.
[b] More than two new IS*10*-containing junction fragments.
[c] Spontaneous deletion.
[d] Two point mutations; two RecA-promoted deletions.

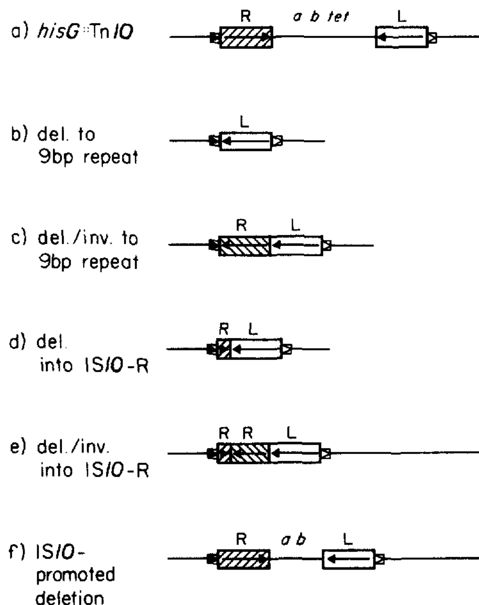

FIGURE 4.—Schematic representation of *hisG9424*::Tn*10* and some of the possible His-revertible Tet^S rearrangement derivatives discussed in the text. The 9 bp *hisG* target site sequence duplicated during Tn*10* insertion is indicated by the small arrow-containing box at each end of the parental insertion.

rangements (HALLING and KLECKNER 1982 and unpublished data), although it is not ruled out that the proximity of IS*10* terminal sequences could also be important.

Neither an IS*10*-promoted adjacent deletion into internal transposon sequences nor an insertion of IS*10* into the *tet* genes was detected. This suggests that the frequency of IS*10*-promoted adjacent deletions is (conservatively) less than 10% the frequency of Tn*10*-promoted deletions and inversion/deletions. The predominance of Tn*10*-promoted events is not due to the presence of a single major Tn*10* insertion hot spot within the IS*10* sequences themselves (KLECKNER, REICHARDT and BOTSTEIN 1979; HALLING and KLECKNER 1982) because at least a third of the observed rearrangements involved several different target sites within the IS sequences.

From one point of view, it should not be surprising that of the revertible Tet^S derivatives are insertions in the *tet* genes. Given the frequency of new IS*10* insertions determined above and the assumption that IS*10* insertions will select targets randomly throughout the genome, insertions into the *tet* genes might easily have been absent in the population of derivatives analyzed. If we (somewhat arbitrarily) take the frequency of IS*10* transposition to be 4×10^{-3} (Table 2) and we assume that the *tet* gene target size is 0.1% of the Salmonella chromosome (2 kb/2000 kb), then *tet*::IS*10* insertions should occur at a frequency of 4×10^{-6}. This corresponds to about 2% of all Tet^S derivatives, and the sample of 150 derivatives examined might easily have contained no such events. Viewed another way, however, the absence of IS*10* insertions is somewhat surprising. The frequency of IS*10* transposition as assayed by Southern blot hybridization is at least ten times higher than the frequency of Tn*10*-promoted deletions and deletion/inversions, and the target size for the two types of events in the analyzed population is about the same, 2–3 kb in each case.

We suggest that the explanation for this apparent conflict is the assumption in the second case that IS*10* transposition and Tn*10*-promoted deletions and deletion/inversions choose target sites in the same way. We propose that IS*10* transpositions do select target sites randomly, but that Tn*10*-promoted deletions and deletion/inversions preferentially use nearby target sites, with the result that the latter two types of events are 'over-represented' in the sample of His-revertible derivatives. Additional data supporting this conclusion are presented in the DISCUSSION.

TABLE 5

Physical analysis of revertible Tet^S rearrangements of *his*G::Tn*10*

Type	KRB class[a]	*his*D expression	Frequency His⁺ revertants	No. analyzed	Type of event:	Deletion					Deletion/Inversion						
						IS*10*-R			IS*10*-L		IS*10*-R					IS*10*-L	
					Target:	A	C	G	A′	Other	A	B	D	E	F	A′	Other
					Site location (bp):	0	130	600	0		0	50	180	330	530	0	
1. Parent																	
	*his*D::Tn*10*	−/+	2×10^{-7}	—		—	—	—	—	—	—	—	—	—	—	—	—
2. Target sites A or A′:																	
	ICi	−/+	2×10^{-7}	15		–	—	—	—	—	13	—	—	—	—	2	—
	IBi and IBii	+(+)	1×10^{-9}	6		5	—	—	1	—	—	—	—	—	—	—	—
3. Other target sites:																	
	ICiv	−/+	2×10^{-8}	1		—	—	—	—	—	—	1	—	—	—	—	—
	ICii	−/+	2×10^{-8}	3		—	—	—	—	—	—	—	3	—	—	—	—
	ICi	−/+	5×10^{-8}	5		—	2	1	—	—	—	—	—	1	—	—	1
	ICiii	−/+	1×10^{-7}	3		—	—	1	—	1	—	—	—	—	1	—	—
			Total:	33													

Target site locations are accurate to ±50 bp. Deletion target sites C and G could be the same as deletion/inversion target sites D and F, respectively. — = not applicable (parent) or none (rearrangements).

[a] KLECKNER, REICHARDT and BOTSTEIN (1979).

The analysis of revertible Tet^S derivatives reveals several other miscellaneous but noteworthy facts. (1) KLECKNER, REICHARDT and BOTSTEIN (1979) assigned the revertible derivatives to different genetic classes based on their His⁺ reversion frequencies and polarity properties; all of the derivatives in the same genetic class turned out to have the same physical structure. (2) Deletion events to either 9 basepair sequence yield a structure which is identical to that resulting from insertion of IS*10* at the *hisG9424* target site (Figure 4b), and these presumptive *hisG*::IS*10* insertions revert at the lowest frequency of any derivative, less than 1% the rate of the parental Tn*10* insertion. This is consistent with previous experiments suggesting that reversion is stimulated by the presence of inverted repeats at the ends of the transposon; the IS*10* insertions have the shortest inverted repeats of any of the Tn*10* derivatives tested (KLECKNER, REICHARDT and BOTSTEIN 1979; FOSTER *et al.* 1981b). Additional support for this view is provided by the series of deletion/inversions ending in IS*10*-Right (Figure 4e). Each such derivative contains the same overall length of transposon material between 9 bp direct repeats; however, as the target site moves farther into IS*10*-Right (top to bottom in Table 4), both the length of the terminal inverted repeat and the frequency of His⁺ revertants increases. (3) Deletion/inversion events to either 9 bp sequence yields a structure containing tandem direct repeats of IS*10* (Figure 4c). The His⁺ reversion frequency of these derivatives is about the same as the parental Tn*10* insertion and 100-fold higher than that of the presumptive IS*10* insertions, suggesting that lengthy direct repeats may also stimulate excision.

DISCUSSION

Relative rates of different transposon-promoted rearrangements: The physical analysis of Tn*10*-, Tn*Gal*-, and IS*10*-promoted DNA rearrangements presented above suggests that the most frequent event is transposition of IS*10*-Right, which is at least ten times more frequent than the next most common events, Tn*10*- or Tn*Gal*-promoted deletions and deletion/inversions. No other simple transposon-promoted event was detected here. Previous experiments have shown that full transposition of Tn*10* and Tn*Gal* in these strains occurs at about 0.1% the rate of IS*10* transposition in minimal medium or about 1% the rate of Tn- promoted rearrangements.

Relative rates of Tn*10* and IS*10* transposition: The 1000-fold difference between the rates of IS*10* and Tn*10* transposition can be ascribed largely but not completely to the difference in length of the two elements. The transposition rates of Tn*10* elements decrease about 40% for every one kilobase increase in transposon length (MORISATO *et al.* 1983). Simple application of this relationship predicts that IS*10* should transpose at 100 times the rate of *nadA*::Tn*10*. The length of Tn*Gal* is unknown.

Several factors could account for the remaining difference. (1) The length-dependence relationship derived for larger elements may not apply to elements as short as IS*10*. (2) Inside and outside IS*10* ends are not genetically identical (ROBERTS *et al.* 1985; D. MORISATO and N. KLECKNER, unpublished data), and an inside IS*10*-Right end may be intrinsically more active for transposition than an outside IS*10*. (3) The rates of transposition of Tn*10* elements, and presum-

ably also of IS*10* elements, vary with the particular site of the starting element, and most of this variation is due to effects of the chromosomal context on the transposition process per se and not to effects of chromosomal location on transposase expression (M. A. DAVIS and N. KLECKNER, unpublished data). The chromosomal contexts of IS*10*-Right and of *nadA*::Tn*10* or *nadA*::Tn*Gal* are not identical. (4) Tn*10*- and Tn*Gal*-promoted deletions and deletion/inversions may be rarer than IS*10* transposition because of some intrinsic mechanistic difference between the two types of events.

IS*10*-R vs. IS*10*-L: Most of the IS*10* transposition events observed in unselected clones were transpositions of IS*10*-Right (Table 2). This preference is probably a reflection of the fact that IS*10*-Right makes functional transposase while IS*10*-Left does not. IS*10* transposase is known to act preferentially on the element encoding it, for two totally different reasons: in part because of intrinsic properties of the protein or its interactions with DNA that limit its ability to move freely in three dimensions (MORISATO *et al.* 1983) and in part because of the way in which IS*10* transposition is regulated by DNA adenine methylation (ROBERTS *et al.* 1985).

Possible medium dependence of IS*10* transposition: The observation of especially high IS*10* transposition in cultures grown in rich medium was somewhat surprising. It has not been observed in previous experiments involving the same strains and virtually identical protocols (RALEIGH and KLECKNER 1984). For this reason we are uncertain as to how much weight to place on the current observations; further experiments are required.

Target site selection during Tn*10* and IS*10*-promoted recombination events: The results presented above raise the possibility that IS*10* transposition events choose target sites randomly throughout the genome while Tn*10*-promoted deletions and deletion/inversions preferentially use adjacent target sites.

There is no other information bearing on the way that IS*10* or Tn*10* selects target sites during transposition. However there are additional indications that Tn*10*-promoted deletion/inversions prefer nearby target. First, the His-revertible deletion/inversions identified above are disproportionately frequent; they comprise about 15% of all deletion/inversions but involve a target region of only 2.6 kb, within Tn*10* itself, out of the roughly 2000 kb in the Salmonella genome. More important, of the majority class (85%) of deletion/inversions that involve target sites outside of Tn*10*, about 90% could be transduced to His$^+$ by a single P22 transducing particle, indicating that they involve target sites less than 35 kb away from the original *hisG*::Tn*10* insertion (KLECKNER, REICHARDT and BOTSTEIN 1979). This leaves only a few percent of deletion/inversion events to more distant target sites. There is no intrinsic reason that inversions should be less than 35 kb in length; substantial inversions of the Salmonella chromosome have been constructed by other methods and shown to be viable (J. ROTH, personal communication).

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