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Genetics

Essential sites at transposon Tn*10* termini

(transposition/DNA–protein interactions/nuclease BAL31)

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ABSTRACT **We describe here point and deletion mutations that define which sequences at the termini of Tn*10* are essential for transposition. We conclude that at least 13 and no more than 27 base pairs of terminal IS*10* sequence are absolutely required at each end. These sequences correspond closely to the terminal inverted repeats of IS*10*. Sequences between base pairs 27 and 70 at each terminus and certain non-IS*10* sequences can also influence transposition, but to a lesser degree. We also describe properties of many function-defective Tn*10* transposition mutants and one exceptional Tn*10* mutant.**

Tn*10* is a 9300-base-pair (bp) tetracycline resistance (Tet^R) transposon whose ends are inverted repeats of IS*10* (Fig. 1). IS*10*-right (IS*10*-R) provides essential transposition functions which act at the termini of Tn*10*. IS*10*-left (IS*10*-L) is a degenerate form of IS*10*-R (1, 2). Previous analysis has shown that all of the sites required for Tn*10* transposition lie in the outer 70 bp at the termini of the element (1). We describe here point and deletion mutations that further define which sequences at these termini are essential. Also, the properties of many function-defective and one exceptional mutant are considered.

MATERIALS AND METHODS

Media and Standard Genetic and Recombinant DNA Techniques. These are described in refs. 1 and 3–6.

Bacteria, Phage, and Plasmids. NK5830 = F′*lacI*^{Q}L8 *pro*/del *lac–pro*$_{X111}$ *arg*$^-$ *su*$^-$ *recA56 thi*$^-$ nalidixic acid-resistant rifampicin-resistant; NK6641 = del *lac–pro*$_{X111}$ *recA56 mal*$^-$ phage λ resistant *strA*; NK5613 = *arg*$^-$ *leu*$^-$ *thr*$^-$ *trp*::Tn*5* *suIII mutD5* is from KD1087 (7); NK6660 = pOX38/*rec*A56 (8); NK5012 = *thr*$^-$, *leu*$^-$, *thi*$^+$, *tonA*$^-$, *lacYI*$^-$, *suII* (also known as C600). Phage λ780 is *b2*::*hisOGD b522 c*I857 Pam80 *nin5* (1); λ814 is λ780 with *hisG9424*::Tn*10** introduced by recombination with pNK214. λ856 is λ780 with Tn*10 del4* HH112 transposed into the *hisG* gene in the same position and orientation as *hisG9424*::Tn*10*. λ1046 is described in ref. 9. Plasmids pNK75, pNK81, pNK82, and pNK289 are described in ref. 1. pNK214 contains the 100-bp *Asu* I to *Bcl* I IS*10*-R junction fragment of pNK81 ligated into pNK81 cut by *Pvu* II and *Bam*HI. pNK133 is pNK75 with the *Bgl* II *tet*^{R} piece of Tn*10* at the *Bgl* II site in *his0*. pNK474 (9) contains the *Pst* I/*Pvu* II "Tac" promoter fragment of pTac12 (10) replacing the *Pst* I/*Bcl* I segment backbone of pNK82. pNK419 and derivatives are described in Figs. 2 and 3.

Isolation of Transposition Mutants. (*i*) *Mutants from* λ::*Tn*10. Plate stocks of repression-proficient integration-defective λ::Tn*10* phages were grown on NK5613 as in ref. 3 with 5% yeast extract present (7). Mutagenized stocks were plated on NK5012. Isolated single plaques were striped with toothpicks onto lawns of NK5012 and incubated at 32°C for 24 hr. These plates were replicated to LB tetracycline (15 μg/ml) plates. After incubation overnight at 32°C, parental λ::Tn*10*s gave many Tet^R colonies per gridded plaque, due to transposition of Tn*10* from the abortively lysogenized λ genomes into the bacterial chromosome. Transposition-defective and tetracycline-sensitive (Tet^S) mutants gave fewer or no colonies. Tet^S structural gene mutants were subsequently eliminated by their failure to give Tet^R transductants by homologous recombination with the resident prophage of a λ lysogen (1). Mutagenized stocks contained 5% clear or semiclear, 1% Tet^S, and 0.5% transposition-defective phages. Nineteen mutants were isolated from λ814 and λ856 (Table 1); eight were from related phages (not shown).

(*ii*) *Mutants from Tn*10 *plasmid.* Purified pNK214 DNA was mutagenized *in vitro* (11) and used to transform the F′*lac–pro* strain NK5830. Individual colonies were screened for ability to give proline-independent (Pro^+) Tet^R exconjugants as follows. Colonies were striped onto LB plates which, after overnight incubation, were replicated onto selective plates (lacking proline but containing streptomycin at 150 μg/ml) spread with 0.5 ml of logarithmic-phase NK6641 recipient culture. After 2 days of incubation, confluent stripes of Pro^+ exconjugants appeared and were then replicated to a minimal streptomycin plate with tetracycline (15 μg/ml) to screen for F′*lac–pro*::Tn*10*s. Wild-type pNK214 gave 50 Pro^+ Tet^R colonies per stripe. Twelve mutants were identified from 3000 screened colonies.

Transposition Assays. The mating-out assay is described in ref. 1. Briefly, single colonies of transposon-containing F′ donor strains were grown into saturated cultures, subcultured, regrown with minimal agitation into exponentially growing conjugation-proficient cultures, and mated for 90 min with NK6641. The mixture was plated on appropriate selective plates to determine the proportion of exconjugant episomes containing transposon insertions. In Fig. 2 and Table 2, 1000 drug-resistant exconjugants per ml of mating mix represents about 10^{-5} transpositions onto the episome *per donor cell*, which corresponds to a transposition rate of 10^{-6} transpositions per cell per generation (9). Donor strains carrying single-copy λ::Tn*10* prophages were obtained as in ref. 1. Infection mating-out assays were the same as simple mating-out assays except that the donor carried a λ^+ prophage and was infected during its final 120 min of growth prior to mating with a λ::Tn*10* phage at multiplicity 16. The infecting phage is repressed by the resident prophage, and the Tn*10* element transposes from λ into F prior to or during the mating period. NK6641 carries a mutation eliminating adsorption of λ, so transposons can enter the recipient strain only by conjugation and not by external infection.

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Abbreviations: bp, base pair(s); kb, kilobase(s); Pro^+, proline-independent; Tet^R and Tet^S, tetracycline resistance (resistant) and sensitivity (sensitive); Kan^R, kanamycin resistance (resistant).

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RESULTS

Point Mutations Affecting Tn*10* Transposition

Transposition-defective mutants of Tn*10* were isolated by two different procedures. (*i*) Integration-defective λ::Tn*10* phages were grown through a mutagenizing (*mutD*) host and individual plaques were screened by a replica plating technique for loss of the ability of Tn*10* to transpose from the abortively lysogenized phage genome into the bacterial chromosome. (*ii*) Plasmids bearing Tn*10* were mutagenized *in vitro* with hydroxylamine and used to transform an F′ host, and individual transformants were screened for altered ability of Tn*10* to transpose from the plasmid into a conjugative F′ episome by a replica-mating assay. Thirty-nine transposition-defective mutants were isolated, 27 by the first approach and 12 by the second.

Mutagenized phage and plasmid genomes carried one of two altered Tn*10* elements (Fig. 1). Both parental elements are deleted for internal portions of IS*10*-L and thus have only one intact transposase gene. Both elements derive directly from *hisG9424*::Tn*10*, the element designated as wild type for studies in this laboratory. *hisG9424*::Tn*10** is a substitution mutant in which a segment containing the left terminus of Tn*10* has been replaced by a segment containing the outermost 70 bp of Tn*10*'s normal right terminus. Since the left and right termini of Tn*10* differ slightly in sequence (2), this manipulation generates a transposon having identical terminal sequences at both ends. *hisG9424*::Tn*10 del*4 is a deletion mutant that retains the normal left end of Tn*10*. Mutants were isolated from λ phages carrying both types of elements, and from pNK214, which carries *hisG9424*::Tn*10**. For comparison with phage mutants, plasmid mutants were subsequently crossed by genetic recombination (1) onto a λ*his* phage, λ780.

Of the 39 transposition-defective mutations analyzed, 4 alter sites required for transposition. They are *cis*-dominant, *trans*-recessive, and themselves make wild-type levels of *trans*-acting transposition functions. Thirty-four mutations affect *trans*-acting transposition function(s). They are recessive to wild type and their defects are complementable in *trans*. One mutation falls into neither class. These conclusions are based on the following genetic tests:

(*i*) The transposition phenotype of each mutant was verified and quantitated by three assays: transposition from a nonreplicating, nonintegrating, nonkilling λ phage into the bacterial chromosome ("λ hop assay") (1), transposition from a chromosomal λ::Tn*10* prophage into an F′ episome ("mating-out assay") (*Materials and Methods*), and transposition from an infecting phage into an F′ episome ("infection/mating-out assay") (Table 1*A*). In all assays, mutant elements transpose at reduced frequencies, ranging from 20% to less than 1% the parental level.

(*ii*) Mutants were tested for their ability to be complemented by a transposase-overproducer plasmid, pNK474, in which transposition functions are expressed from the strong *ptac* promoter (Table 1*B*). The high level of transposition functions produced by this plasmid increases transposition of a parental single-copy Tn*10* element roughly 50-fold (9). In the presence of this plasmid, function-defective mutants are fully complemented and transpose at the same rate as their

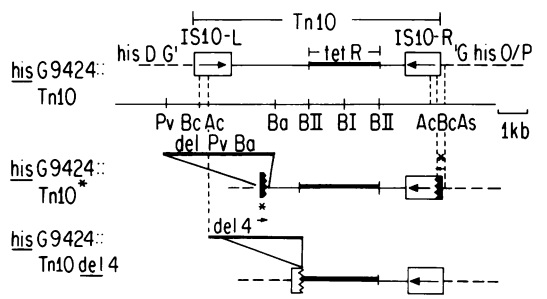

FIG. 1. Physical maps of the *hisG*::Tn*10* insertion defined as wild type in this laboratory and two variants constructed *in vitro*. In Tn*10**, IS*10*-L is completely deleted (*del* Pv Ba), and replaced with a small segment (*) containing the terminus of IS*10*-R in appropriate orientation as shown. Tn*10-del*4, deleted between the indicated *Acc* I and *Bgl* II sites, retains the normal left terminus. The parental insertion and *del*4 are described in ref. 1, and Tn*10**, on pNK214, in *Materials and Methods*. Restriction endonuclease sites: Pv, *Pvu* II; Bc, *Bcl* I; Ac, *Acc* I; Ba, *Bam*HI; BI, *Bgl* I; BII, *Bgl* II; As, *Asu* I (not all sites are shown); Cl, *Cla* I. kb, Kilobase pair.

Table 1. Transposition and complementation of mutant λ::Tn*10* phages in infection/mating-out assays

Mutation on λ::Tn*10*†	Exp. A. λ::Tn*10* + *del*16 *del*17 *kan*^{R} Tet^R	Exp. A. λ::Tn*10* + *del*16 *del*17 *kan*^{R} Kan^R	Exp. B. λ::Tn*10* + pNK474 Tet^R
Controls			
Tn*10 del*16 *del*17-(λ809)‡	0	1§	NT
WT Tn*10**(λ814)	126	36	826
No phage	0	1§	NT
Class I, function-defective (*n* = 34)			
822	0	2	719
908¶	0	1	334
977	1	0	784
818	4	2	915
823	4	8	986
973	9	5	815
820	27	14	547
829	28	6	547
984	34	20	530
Class II, site-defective (*n* = 4)			
980	0	68	8
981	2	49	3
910¶	0	225	0
913¶	4	177	0
Class III, other (*n* = 1)			
G8 (also known as 830)	0	2	83

Transposition and complementation of/by Tn*10* mutants was assayed by infecting a *recA*$^-$ F$^+$ strain, NK6660(λ) with λ::Tn*10* phages, allowing time for transposition events to occur, and measuring the frequency of transpositions into F by mating the infected culture with a suitable *recA*$^-$ λ-resistant recipient, NK6641. In *A*, the infected strain contains pNK289 (1), which carries a function-defective deletion mutant of Tn*10* marked with *kan*^{R} genes. Tet^R exconjugants are transpositions of the Tn*10* element itself; kanamycin-resistant (Kan^R) exconjugants are transpositions of the Kan^R deletion variant in response to functions provided by the λ::Tn*10* phage. In *B*, the infected strain carries pNK474, which overproduces IS*10* transposition functions (ref. 9 and text). Tet^R exconjugants are transpositions of the input Tn*10* element promoted by these functions. Data presented are numbers of Tet^R or Kan^R exconjugants per 0.3 ml (Exp. A) or 0.05 ml (Exp. B) of mating mix. WT, wild type; NT, not tested.

†Total number of mutants in each class indicated in parentheses; representative function mutants shown. All mutants listed in this table were isolated from λ814 except for three (¶) isolated from λ856, which contains *hisG9424*::Tn*10 del*4 HH112, a mutant whose transposition frequency is twice that of Tn*10** in this assay.

‡λ809 carries *hisG*::Tn*10 del*16 *del*17 (1), which has undergone deletion of all transposition functions.

§Residual Kan^R exconjugants are due to non-IS*10*-mediated illegitimate recombination.

corresponding wild-type parent. Transposition of the four site mutants is less than 5% of the parental level, and one atypical mutant, G8, transposes at 20% of the wild-type level. Control experiments show that inefficient complementation of the latter five mutants is not due to *trans*-dominance of the mutations on the ability of pNK474 to provide transposition functions (not shown) and therefore reflects *cis*-dominant transposition defects.

(*iii*) Each mutant was tested for its ability to complement a plasmid-borne function-defective Tn*10* deletion derivative, Tn*10 del*16 *del*17 *kan*^{R} (Table 1*A*). The function mutants are all unable to complement this element, while the four site mutants complement as efficiently as their wild-type parents. The exceptional G8 mutant differs from the other *cis*-dominant mutants: it fails to provide complementing functions.

The DNA sequence changes corresponding to the five *cis*-dominant mutations are shown in Fig. 2. All four site mutations occur in the outermost 13 bp of Tn*10*. Mutations 980 and 981 are transitions at bp 9 and 13 of IS*10*-L, respectively, while 913 is an insertion of a C·G base pair between bp 8 and 9 of IS*10*-L. Mutation 910 is identical to 981 but is in IS*10*-R. The G8 mutation is a single base transition at bp 151. The G8 phenotypes result primarily from an alteration in IS*10*'s regulatory promoter, pOUT (ref. 12 and below).

Deletion Mutations in Tn*10* Termini

The DNA sequences that are structurally required for Tn*10* transposition are further defined by a series of deletion mutations that extend from inside Tn*10* towards one outside end. Deletion mutants were isolated from a parental transposon consisting of a segment of DNA containing ColE1 and *lacZ*, *trpA*, and *tet*^{R} genes flanked by inverted repeats of a DNA segment containing the outer 70 bp of IS*10*-R (pNK419, Fig. 3). The transposon has a unique *Bam*HI site at the junction between one terminus and the internal ColE1 sequences. In agreement with previous observations (1), this parental element has all essential sites intact. When complemented, it transposes at approximately the rate expected from its length and from the known efficiency of IS*10* complementation (9).

Elements with BAL31 deletions that eliminate DNA on both sides of the unique *Bam*HI site of pNK419 were isolated, the DNA sequences around the deletion junctions were determined, and the elements with deletions were tested for transposition when complemented (Fig. 2). Two deletion mutants that retain 13 or fewer bp of terminal IS*10* sequence at the deleted end are grossly defective and transpose at less than 1% the parental level. Eight deletion mutants that retain 27 or more bp transpose at 50–100% the parental level, with pNK561 (28 bp) and pNK556 (27 bp) reproducibly the lowest. The variation in transposition frequency among the different mutants is probably due to the differences in IS*10* sequences and not to changes in adjacent ColE1 DNA. Mutants with identical IS*10* sequences and different ColE1 sequences (e.g., pNK555 and -562) transpose at the same frequencies, while mutants with the same ColE1 sequences and different IS*10* sequences (e.g., pNK555 and -556) do not. The deletions described in Fig. 2 identify only those sequences whose presence is required at both ends of the transposing segment, because the mutant elements all retain one wild-type terminus. In this set of elements, all such sequences lie within the outer 27 bp of Tn*10*.

Further information about sequences from bp 27 to 70 is provided by analysis of transposon constructions in which Tn*10 tet*^{R} genes alone are flanked by combinations of deleted or 70-bp termini (Fig. 3, Table 2). When paired with a 70-bp terminus, the deletion mutations have the same effect on transposition in this construction as in the construction where they were isolated. Deletions leaving 62 or 42 bp at one end reduce transposition to 2/3 the 70 × 70 level, and a deletion leaving 27 bp has a more severe effect, reducing transposition to 1/4 the 70 × 70 level.

In elements containing deletion mutant termini at both ends, the two deletion mutations are multiplicative in all cases but one (below), suggesting that the activity of each

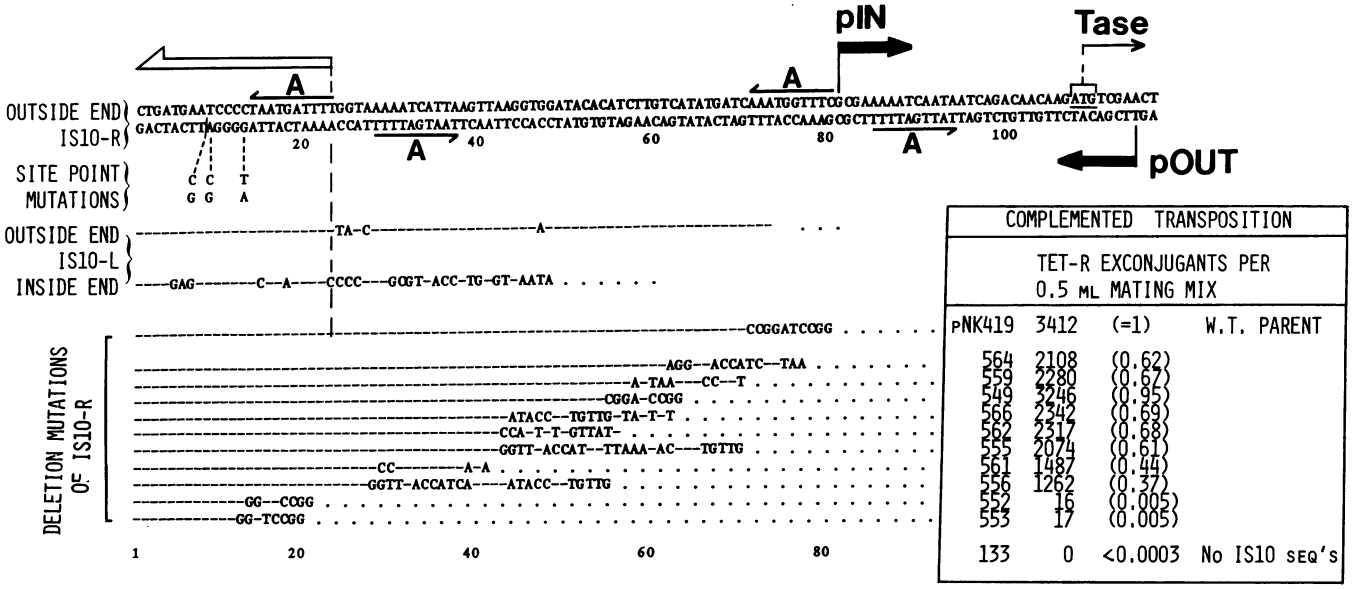

FIG. 2. DNA sequences at the outside end of IS*10*-R and sequences and properties of related IS*10* termini. Transcription startpoints of pIN and pOUT promoters, the start of the transposase (Tase) coding region, a 10-bp repeated element (sequence A), and the terminal inverted repeat segment common to the inside and outside ends of IS*10*-R are indicated. Point mutant 913 is an insertion of a C·G base pair between bp 8 and 9 of IS*10*-L; 910 is a transition at bp 9 in IS*10*-R, and 980 and 981 are transitions at bp 9 and 13, respectively, in the IS*10*-R terminal sequences at the left end of Tn*10**. Sequences at IS*10*/non-IS*10* junctions of pNK419 and deletion derivatives and complemented transposition of these elements in a mating-out assay are also shown. Host donor strain for mating-out was NK6660 containing as prophage λ1046, which bears a pTac–transposase overproducer fusion; recipient was NK6641. pNK133 is a control plasmid containing the *tet*^{R} genes of Tn*10* but no IS*10* material. Dashes are positions where variant sequences are identical to the sequence of IS*10*-R.

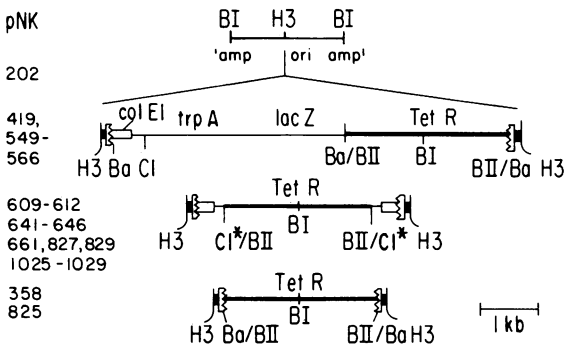

FIG. 3. (*i*) pNK202 is the pBR322-derived plasmid pGL101 (13, 14) with a *Hin*dIII linker inserted between filled-in *Hin*fI sites at bp 2374 and 2449. (*ii*) pNK419 was constructed in two steps. First, pNK357 (not shown) was made by inserting at the *Hin*dIII site of pNK202 a ColE1/*trpA–lacZ* W205 *Bam*HI fragment from pRB205 (9, 15) flanked by inverted repeats of a *Hin*dIII/*Bam*HI segment containing the outer 70 bp of IS*10*-R plus an adjacent 36 bp of λ *cI* gene sequence. This IS*10*/*cI* segment is from the right junction of λ*cI*171::Tn*10* (16) with a *Bam*HI linker inserted at a *Bcl* I site at bp 70 of IS*10*. pNK419 is derived from pNK357 by insertion of the 2800-bp *Bgl* II *tet*^{R} segment of Tn*10* into the "right-hand" *Bam*HI site. This manipulation leaves a unique *Bam*HI site at the junction between IS*10* and ColE1 material. (*iii*) For pNK549–566, pNK419 was cleaved at its unique *Bam*HI site, treated for 2–8 min with BAL31 [generously supplied by Legerski *et al.* (17)], and ligated in the presence of *Bam*HI linkers. Only pNK549, -552, and -553 incorporated a linker. (*iv*) pNK609–612 and -661 were made by inserting at the *Hin*dIII site of pNK202 the *Bgl* II *tet*^{R} fragment (above) flanked by inverted repeats of the *Hin*dIII/*Cla* I terminus segments from pNK419 or derivatives. [Preparatory to this construction, the *Cla* I sites in pNK419 etc. were changed to either *Bgl* II (pNK419) or *Bam*HI (deletions) sites, denoted in the figure as Cl*.] The *Hin*dIII/*Cla* I* terminus fragments of pNK609, -611, -612, and -661 are from pNK555, -556, -564, and -419, respectively. (*v*) pNK641–646, -827, -829, and -1025–1029 are analogous in structure to pNK609 etc., except they have different deletions at their left and right termini. They were constructed from pNK609, -611, -612, and -661 by ligation of purified *Bgl* I fragments as follows: pNK641 = left end (L) of pNK611 + right end (R) of pNK609; pNK642 = pNK612-L + pNK609-R; pNK643 = pNK609-L + pNK611-R; pNK645 = pNK611-L + pNK612-R; pNK646 = pNK609-L + pNK612-R; pNK827 = pNK612-L + pNK661-R; pNK829 = pNK661-L + pNK609-R; pNK1025 = pNK612-L + pNK611-R; pNK1026 = pNK609-L + pNK661-R; pNK1027 = pNK661-L + pNK611-R; pNK1028 = pNK611-R + pNK661-R; pNK1029 = pNK661-L + pNK612-R. (*vi*) pNK358 is derived from pNK357 by replacing the *trp–lac Bam*HI fragment with the *Bgl* II *tet* fragment. (*vii*) pNK825 is pNK202 having at its *Hin*dIII site the *Bgl* II *tet*^{R} fragment flanked by inverted repeats of the *Hin*dIII/*Bam*HI terminus of pNK549. These terminus segments contain only 54 bp of IS*10* sequences and no ColE1 material, because there is a *Bam*HI linker at the BAL31 deletion junction of pNK549. All plasmids contain the *tet*^{R} genes in the same orientation relative to the plasmid backbone. Restriction site code as in Fig. 1.

terminus is affected independently by its deletion mutation. Thus, 62 × 62, 42 × 42, 42 × 62, and 62 × 42 elements transpose at 40–50% (= 4/9) of the 70 × 70 level, and 27 × 42, 27 × 62, 42 × 27, and 62 × 27 elements transpose at 10–20% (= 1/6) of the 70 × 70 level. The exceptional situation is an element containing 27-bp termini at both ends. A 27 × 27 element reproducibly transposes at 1% of the 70 × 70 level rather than the expected 6% (= 1/16). This result suggests that it is more important that the information between bases 27 and 42 be present at one end or the other than that it be present at both ends.

Non-IS*10* DNA sequences can also influence terminus activity. Transposons that carry a segment of IS*10* plus adjacent ColE1 material from pNK419 at both termini transpose less efficiently than analogous derivatives lacking the ColE1 DNA. A transposon containing 70-bp IS*10* termini with the inhibitory segment at both ends (pNK661) transposes at 1/3 the frequency of an otherwise identical element lacking that segment (pNK358). Similarly, elements containing two 42-bp termini or two 62-bp termini with the inhibitory segment at both junctions (pNK609, pNK612) transpose at 20% the frequency of a transposon (pNK825) that contains two 54-bp termini and no inhibitory segment. Plasmids are described in Fig. 3 and Table 2.

Table 2. Complemented transposition of Tn*10* elements with deletions into both termini: Tet^R exconjugants/0.1-ml mating mix

IS*10* bp at right end	IS*10* bp at left end			
	70	62	42	27
70	456	227	324	118
62	309	181	224	85
42	316	178	184	71
27	105	57	42	2

Transposons carrying different combinations of deletion mutations at their termini were tested for transposition (when complemented) by a mating-out assay as described in the legend of Fig. 2 and *Materials and Methods*. Plasmids used (Fig. 3): 70 × 70 = pNK661; 62 × 70 = pNK827; 42 × 70 = pNK1026; 27 × 70 = pNK1028; 70 × 62 = pNK1029; 62 × 62 = pNK612; 42 × 62 = pNK642; 27 × 62 = pNK645; 70 × 42 = pNK829; 62 × 42 = pNK1025; 42 × 42 = pNK609; 27 × 42 = pNK643; 70 × 27 = pNK1027; 62 × 27 = pNK646; 42 × 27 = pNK641; and 27 × 27 = pNK611.

Further Analysis of Function Mutants

Each function-defective mutant was tested not only for its ability to be complemented by a transposase overproducer plasmid (above) but for its ability, when present on a λ::Tn*10* prophage, to be complemented by a differentially marked (chloramphenicol resistance) single-copy Tn*10* element present elsewhere in the same chromosome. Complementation of all mutants was very inefficient: transposition was not detectably increased by the chloramphenicol resistance helper (not shown). Thus, IS*10* transposition functions are preferentially *cis*-acting in the formal genetic sense. Morisato *et al.* (9) have shown that IS*10* transposition functions work more efficiently on transposon ends near the gene from which they are synthesized than on ends located farther away. This *cis* action has precluded complementation tests among the different function-defective mutants. We also note that because transposase is *cis*-acting, *trans*-dominant mutations in the transposase gene, analogous to *lacI*$^{-d}$ (18), should not occur; and in fact, none were isolated.

An Exceptional *cis*- and *trans*-Dominant Mutation Alters pOUT

The G8 mutation reduces Tn*10* transposition and expression of IS*10* transposition functions (Table 1*A*) and causes a *cis*-dominant defect even when transposition functions are provided from an overproducer plasmid (Table 1*B*). This defect is not due to interference by a defective, *cis*-acting transposase protein, because it is also observed in G8 derivatives with deleted IS*10* coding sequences (not shown). Single-copy complementation tests (not shown) reveal another G8 phenotype: a single prophage copy of λTn*10**-G8 is *trans*-dominant on transposition of a marked (chloramphenicol resistance) wild-type transposon, and it interferes with the ability of that element to express complementing transposition functions. DNA sequence analysis (above) places the G8 mutation in the −35 region of the regulatory promoter of IS*10*, pOUT. Other experiments (12) show that G8 increases expression from pOUT both *in vivo* and *in vitro* and suggest

that the *trans*-dominant effect of G8 on expression from a wild-type element is due to the ability of pOUT transcript to act in *trans* to inhibit translation of the transposase gene. The *cis*-dominant defect in G8 transposition in the presence of complementing functions may reflect an inhibition of transposition in *cis* by transcription from pOUT. The failure of G8 itself to express transposition functions is not understood, but it could be due to the change in the amino acid sequence of the transposase protein, to interference between pIN and pOUT at the transcriptional level, or to both (ref. 15 and unpublished data).

DISCUSSION

The results presented above suggest that the outer ≈27 bp of Tn*10* contain sites that are absolutely essential for Tn*10* transposition. Point mutations at bp 9 and 13 and deletion mutations leaving 12 or 13 bp at one end confer drastic *cis*-dominant transposition defects, even when the transposon has a normal terminus at the other end. Deletion mutations leaving 27 or more bp are much less defective. This functional division corresponds well to the DNA sequence organization of IS*10*. The outer 23 bp of Tn*10* are present as nearly perfect inverted repeats at the inner termini of the two IS*10* sequences. Furthermore, this outer 23 bp can be subdivided into two parts. Base pairs 1–13 is a unique sequence found only at the two termini of IS*10*, while bp 14–23 is a sequence that occurs five times at or near the ends of IS*10* (sequence A in Fig. 2 and ref. 2). Thus, it is reasonable that the most important sites for transposition are all in the outer 27 bp of IS*10*, with the outer 13 bp being especially important. The region between bp 13 and 27 has not been further dissected.

Deletion analysis suggests that bp 27–70 are also important for transposition, at least in these artificial constructions. Constructions containing 27-bp termini at both ends transpose at only 1% the parental (70 × 70) level. The region from bp 27 to bp 70 appears to be complex. Deletion of bp 42–70 results in small and independent decreases in the activity of either or both termini in a constructed transposon. Deletion of bp 27–70 results in a more severe defect, suggesting an additional role of bp 27 to 42. Furthermore, when present at both ends, such deletions reduce transposition much more than expected from their effect when paired with a 70-bp terminus. Thus, at least in these transposons, it seems to be more important to have the information from bp 27–42 present in at least one terminus and somewhat less important to have it at the second terminus as well.

Base pairs 27–70 of IS*10* may encode auxiliary sites that directly facilitate, supplement, or modify interactions in the essential bp 1–27. This region contains an additional copy of sequence A, which might be important. Alternatively, these sequences may influence terminus activity only indirectly. For example, these sequences are probably important for termination of any transcription that happens to proceed from inside the transposon outwards towards the termini. pOUT transcription terminates between bp 20 and bp 30 *in vitro* (refs. 12 and 15; unpublished data); *in vivo*, a pOUT transcript has been identified with its 3′ end localized to this region (F. Schmidt, personal communication); and bp 26–34 conform to the consensus sequence for *boxA*, which is likely to be at the site at which NusA protein interacts with RNA, DNA, or both (ref. 19; D. Friedman, personal communication). If high levels of transcription from internal promoters were inhibitory to transposition, as suggested by the *cis*-dominant noncomplementable defect of the G8 mutation, deletions in bp 27–70 might decrease transposition because they allow continuation of readthrough transcription outwards from internal (in these constructions, tet^R gene) promoters. Further experiments are needed to decide whether bp 27–70 are as important for transposition of an intact IS*10* or Tn*10* element as they are in the transposon constructs analyzed here. In the above case, for example, wild-type IS*10* may have other mechanisms for blocking readthrough transcription before it reaches the outer terminus.

Non-IS*10* sequences adjacent to the termini could also inhibit transposition either directly or indirectly. The inhibitory effect does not depend upon the particular nature of the IS*10*/ColE1 junction sequences. One simple possibility is that the ColE1 material contains a new promoter whose activity mimics the inhibitory effect of pOUT in Tn*10*-G8.

Many other insertion sequences, such as IS*50*, Tn*3*, and IS*903*, have short terminal inverted repeats similar to those in IS*10* (20). In some cases, there is evidence that these repeats are structurally required for transposition and that only a small sequence at each end of the element is important. A small insertion/deletion in one of the 38-bp terminal repeats of Tn*3* causes a noncomplementable transposition defect (21). A series of deletions within Tn*5* limit the important sequences for transposition to the outermost 18 bp (22, 23).

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