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Transposon Tn7

cis-Acting Sequences in Transposition and Transposition Immunity

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We have identified and characterized the *cis*-acting sequences at the termini of the bacterial transposon Tn7 that are necessary for its transposition. Tn7 participates in two kinds of transposition event: high-frequency transposition to a specific target site (*attTn7*) and low-frequency transposition to apparently random target sites. Our analyses suggest that the same sequences at the Tn7 ends are required for both transposition events. These sequences differ in length and nucleotide structure: about 150 base-pairs at the left end (Tn7L) and about 70 base-pairs at the right end (Tn7R) are necessary for efficient transposition. We also show that the ends of Tn7 are functionally distinct: a miniTn7 element containing two Tn7R ends is active in transposition but an element containing two Tn7L ends is not. We also report that the presence of Tn7's *cis*-acting transposition sequences anywhere in a target replicon inhibits subsequent insertion of another copy of Tn7 into either an *attTn7* target site or into random target sites. The inhibition to an *attTn7* target site is most pronounced when the Tn7 ends are immediately adjacent to *attTn7*. We also show that the presence of Tn7R's *cis*-acting transposition sequences in a target replicon is necessary and sufficient to inhibit subsequent Tn7 insertion into the target replicon.

1. Introduction

Transposable elements are discrete DNA segments that can move from one genetic location to another. The bacterial transposon Tn7 (Fig. 1(a); Barth *et al.*, 1976), which carries resistance to trimethoprim and to streptomycin and spectinomycin, can participate in two types of transposition event: high-frequency insertion into specific target sites in the chromosomes of many different bacteria and low-frequency insertion into many other random target sites (for a review, see Craig, 1989). The specific site of Tn7 insertion into the *Escherichia coli* chromosome, *attTn7*, lies at about minute 84 between *phoS*, a gene involved in phosphate transport, and *glmS*, a gene involved in cell wall biosynthesis (Barth *et al.*, 1976; Lichtenstein & Brenner 1981, 1982; Walker *et al.*, 1984). Tn7 inserts into *attTn7* in a unique orientation (Lichtenstein & Brenner, 1981). When Tn7 occupies *attTn7* (*attTn7* :: Tn7), the left end of Tn7 (Tn7L), which is adjacent to its drug resistance genes, is always proximal to *phoS*, and the right end of Tn7 (Tn7R), which is adjacent to its transposition genes, is always proximal to *glmS*. In addition to site and orientation-specific transposition to *attTn7*, Tn7 also transposes at low frequency to many different plasmid and chromosomal sites (Barth & Grinter, 1977; Barth *et al.*, 1978; Moore & Krishnapillai, 1982; K. Kubo & N. Craig, unpublished results).

Transposable elements carry two kinds of information to promote their transposition: (1) genes encoding transposition proteins and (2) DNA sites at the element termini that participate directly in transposition (for reviews, see Grindley & Reed, 1985; Craig & Kleckner, 1987). Tn7 contains five transposition genes: *tnsA*, *tnsB*, *tnsC*, *tnsD* and *tnsE* (Fig. 1(a); Rogers *et al.*, 1986; Waddell & Craig, 1988). These genes provide two distinct but overlapping transposition pathways (Rogers *et al.*, 1986; Waddell & Craig, 1988, K. Kubo & N. Craig, unpublished results). *tnsABC+tnsD* promote high-frequency transposition to *attTn7* and, when *attTn7* is unavailable, low-frequency transposition to other sites structurally related to *attTn7*. *tnsABC + tnsE*

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promote low-frequency transposition to many different sites unrelated to *attTn7* and to each other. Thus, Tn7 engages in transposition to both specific target sites and to random target sites.

Previous studies have shown that *cis*-acting transposition sequences lie at the ends of Tn7 (Hauer & Shapiro, 1984; Smith & Jones, 1984; Ouartsi *et al.*, 1985; Barry, 1986; Rogers *et al.*, 1986). In this report, we define and characterize the *cis*-acting transposition sequences at the Tn7 ends. We show that about 150 bp† at the terminus of Tn7L and at least 70 bp at the terminus of Tn7R participate directly in transposition. Thus, considerable sequence information at each end of Tn7 beyond this element's terminal 30 bp inverted repeats (Lichtenstein & Brenner, 1982; Gosti-Testu & Brevet, 1982) is required for transposition. We also show that the ends of Tn7 are functionally different.

The *cis*-acting transposition sequences of the transposons Tn3 and bacteriophage Mu have been shown to provide another interesting activity, transposition immunity (Lee *et al.*, 1983; Reyes *et al.*, 1987; Adzuma & Mizuuchi, 1988). Transposition immunity is the ability of a transposable element present in a target DNA molecule to reduce substantially the frequency of subsequent insertion of another copy of the element into this target molecule. The same sequences that participate directly in transposition are necessary and sufficient to provide transposition immunity (Lee *et al.*, 1983; Huang *et al.*, 1986; Adzuma & Mizuuchi, 1988). Thus transposition immunity is fundamentally related to the process of transposition itself. The phenomenon of transposition immunity is an interesting example of "action at a distance", as short DNA segments containing the transposon ends can render large target molecules approaching 100 kb in size immune to further transposon insertion anywhere within the target molecule.

Hauer & Shapiro (1984) have shown that the presence of Tn7, or large DNA segments containing the Tn7 ends, in a target plasmid reduces the frequency of subsequent Tn7 insertion into these targets, i.e. that Tn7 displays transposition immunity. In this report, we characterize Tn7 transposition immunity and show that it is active in both the *tnsABC+tnsD* and the *tnsABC+tnsE* transposition pathways. We also present evidence that the sequences in Tn7R that are necessary for efficient Tn7 transposition can provide transposition immunity to a target DNA molecule.

2. Materials and Methods

(a) *Media, chemicals and enzymes*

LB broth and agar were as described by Miller (1972) except that 1 mg glucosamine was added/ml agar. "Iso-sensitest Agar" (Oxoid) was used with trimethoprim. Supplements were: carbenicillin (100 μg/ml), chloramphenicol (30 μg/ml), gentamicin (10 μg/ml), kanamycin (50 or 100 μg/ml), rifampicin (100 μg/ml), tetracycline (5 or 20 μg/ml) and trimethoprim (100 μg/ml). DNA-modifying enzymes were obtained from commercial sources and used as recommended by the manufacturer.

(b) *Bacterial strains*

NLC51 is *E. coli* F⁻ *araD*139 Δ (*argF-lac*) U169 *rps*L150 *relA*1 *ftbB*5301 *deoC*1 *ptsF*25 *rbsR* *val*^{R} *recA*56; LA3 is NLC51 *attTn7* :: Tn7 (McKown *et al.*, 1987). The Tn7 derivative used in this work, Tn7S, contains an IS*1* element inserted near its antibiotic resistance determinants (Fig. 1(a)); the transposition properties of Tn7S are indistinguishable from those of the canonical Tn7 (Hauer & Shapiro, 1984). CW51 is *E. coli* F⁻ *ara*⁻ *arg*⁻ *lac* *pro*XIII *recA*56 Nal^R Rif^R (Waddell & Craig, 1988).

(c) *Manipulation and analysis of DNA*

Plasmid growth, isolation, transformation and restriction enzyme analysis were performed as described by Maniatis *et al.* (1982). Cloning procedures were as described by Maniatis *et al.* (1982) except that DNA fragments contained in slices excised from low-melting temperature agarose gels (Sea Plaque) were used directly in assembly of recombinant molecules as described by Struhl (1983). Phage lambda growth was performed as described by Maniatis *et al.* (1982).

(d) *Plasmids*

(i) attTn7 *plasmids*

In *attTn7*, the middle base of the 5 bp chromosomal sequence duplicated upon Tn7 insertion is designated 0, sequences extending leftward toward *phoS* are given "−" numbers and sequences extending rightward toward *glmS* are given "+" numbers. pRM2 is a Cb^R pUC18 derivative containing *attTn7* −342 to +165. pKAO3 is a Cb^R pUC18 derivative containing *attTn7* −52 to +64 (McKown *et al.*, 1988).

(ii) tns *plasmids*

pCW4, pCW4 :: miniMu78 and pCW4 :: miniMu72 are Tc^R pACYC184 derivatives containing the *tns* genes (Waddell & Craig, 1988). pCW4 provides *tnsABCDE*, pCW4 :: miniMu78 provides functional *tnsABC+tnsD* and pCW4 :: miniMu72 provides functional *tnsABC+tnsE*.

(iii) *Plasmids containing miniTn*7L1900 × R537 *and miniTn*7L166 × R199

pRM2 :: miniTn*7L1900* × *R537* was constructed by *in vivo* transposition of the Cm^R miniTn7 derivative Tn7S :: Tn*9* Δ *Pst*I (Hauer & Shapiro, 1984) into *attTn7* of pRM2 (Fig. 1(b); McKown *et al.*, 1988).

pLA1 contains *attTn7* :: miniTn*7L166* × *R199*; this miniTn7 element contains a Km^R marker (Fig. 1(c); McKown *et al.*, 1987). It was obtained from pRM2 :: miniTn*7L1900* × *R537* by digestion with *Hpa*I and *Bal*I which cut in Tn7L and Tn7R, respectively (Lichtenstein & Brenner, 1982), ligation in the presence of *Sal*I linker (Pharmacia) and subsequent introduction of a fragment with terminal *Sal*I sites encoding Km^R from pUC-4K (Pharmacia).

pKAO3 :: miniTn*7L166* × *R199* contains *attTn7* :: miniTn*7L166* × *R199* (Fig. 1(d); McKown *et al.*, 1988).

† Abbreviations used: bp, base-pairs; kb, 10^3 base-pairs; Cb, carbenicillin; Cm, chloramphenicol; Gen, gentamicin; Km, kanamycin; Rif, rifampicin; Tc, tetracycline; Tp, trimethoprim; nt, not tested; s.e.m., standard error of the mean.

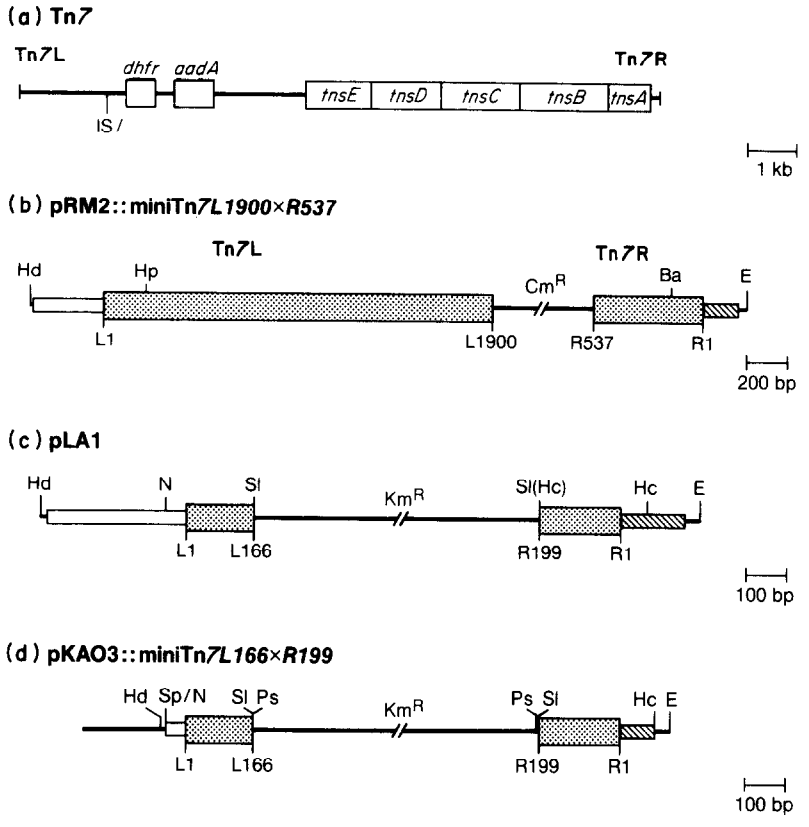

Figure 1. Structure of Tn*7* and plasmids containing miniTn*7* elements. (a) Tn*7*. *dhfr* encodes a dihydrofolate reductase (Fling & Richards, 1983; Simonsen *et al.*, 1983) that provides resistance to trimethoprim, *aadA* encodes a 3″(9)-*O*-nucleotidyltransferase (Fling *et al.*, 1985) that provides resistance to streptomycin and spectinomycin, and *tnsA*, *tnsB*, *tnsC*, *tnsD*, *tnsE* are Tn*7*'s transposition genes (Rogers *et al.* 1986; Waddell & Craig, 1988). The left end of Tn*7* (Tn7L) and the right end of Tn*7* (Tn7R) are indicated. The position of IS*1* in Tn7S is indicated (Hauer & Shapiro, 1984). In (b) to (d), the segments shown are contained within the polylinkers of the indicated plasmids. The Tn7L and Tn7R end segments are shown by stippled boxes and their boundaries are numbered. The Tn*7* end segments are flanked by the sequences that flank the Tn*7* ends in chromosomal *attTn7*. The chromosomal sequences which flank L1 and extend leftward towards *pho*S are indicated by an open box and the chromosomal sequences which flank R1 and extend rightward are indicated by a hatched box. The nucleotide sequence information required for *attTn7* target activity lies within the hatched boxes (Gringauz *et al.*, 1988). Cm^R and Km^R designate segments within the miniTn*7* elements that encode chloramphenicol resistance and kanamycin resistance. Restriction enzyme sites relevant to this work are shown: Ba, *Bal*I; E, *Eco*RI; Hc, *Hin*cII; Hd, *Hin*dIII; Hp, *Hpa*I; N, *Nla*III; Ps, *Pst*I; Sl, *Sal*I; and Sp, *Sph*I. (b) pRM2::miniTn*7L1900* × *R537*. (c) pLA1 (pRM2::miniTn*7L166* × *R199*). (d) pKAO3::miniTn*7L166* × *R199*.

pEM-1 contains miniTn*7L166* × *R199* flanked by *E. coli* chromosomal sequences, about 1600 bp flanking Tn7L and about 400 bp flanking Tn7R. It was obtained by *tnsABC*+*tnsE*-dependent transposition of miniTn*7L166* × *R199* to the *E. coli* chromosome at site EC-18 (*tnsE* chromosomal) and subsequent cloning of the *Eco*RV chromosomal fragment containing the miniTn*7* element into the *Hin*cII site of pUC19 (K. Kubo, R. Bainton & N. Craig, unpublished results).

(iv) *Plasmids containing deleted Tn7L end segments used in the assembly of miniTn7 elements*

Plasmids containing Tn7L end segments are shown in Fig. 2(a). pLA26 contains Tn7L166 flanked at its L1 terminus by *E. coli* chromosomal sequences that lie to the left (towards *phoS*) of the specific point of Tn*7* insertion in *attTn7*. It was obtained by treating the Tn7L-containing *Hin*dIII–*Pst*I fragment from pKAO3::miniTn*7L166* × *R199* with Klenow fragment and inserting it into the *Sma*I site of pUC18 with chromosomal sequences adjacent to vector p*lac*.

pLA30 (L149), pLA32 (L94), pLA57 (L75) and pLA34 (L23) contain shorter Tn7L segments obtained by restriction enzyme digestion. The Tn7L-containing *Pvu*II fragment from pLA26 was isolated by electroelution, digested with *Mae*I, *Hga*I, *Mae*III or *Mnl*I and treated with Klenow fragment when necessary. The *Mae*I–*Pvu*II, *Hga*I–*Pvu*II and *Mnl*I–*Pvu*II fragments containing Tn7L terminal sequences were introduced into the *Sma*I site and the *Mae*III–*Pvu*II fragment was introduced into the *Hin*cII site of pUC18. In all cases, the chromosomal sequences flanking the Tn7L segment are adjacent to vector p*lac*.

pLA40 (L108) was constructed by isolation by electoelution of the Tn7L-containing *Hin*dIII–*Pst*I fragment from pKAO3::miniTn*7L166* × *R199* and digestion of this fragment with *Alu*I. The resulting *Alu*I–*Hin*dIII fragment containing Tn7L was inserted into *Hin*dIII–*Hin*cII site of pUC18.

(v) *Plasmids containing deleted Tn7R end segments used in the construction of miniTn7 elements*

Plasmids containing Tn7R segments are shown in Fig. 2(b). pLA28 contains Tn7R199 flanked at its R1

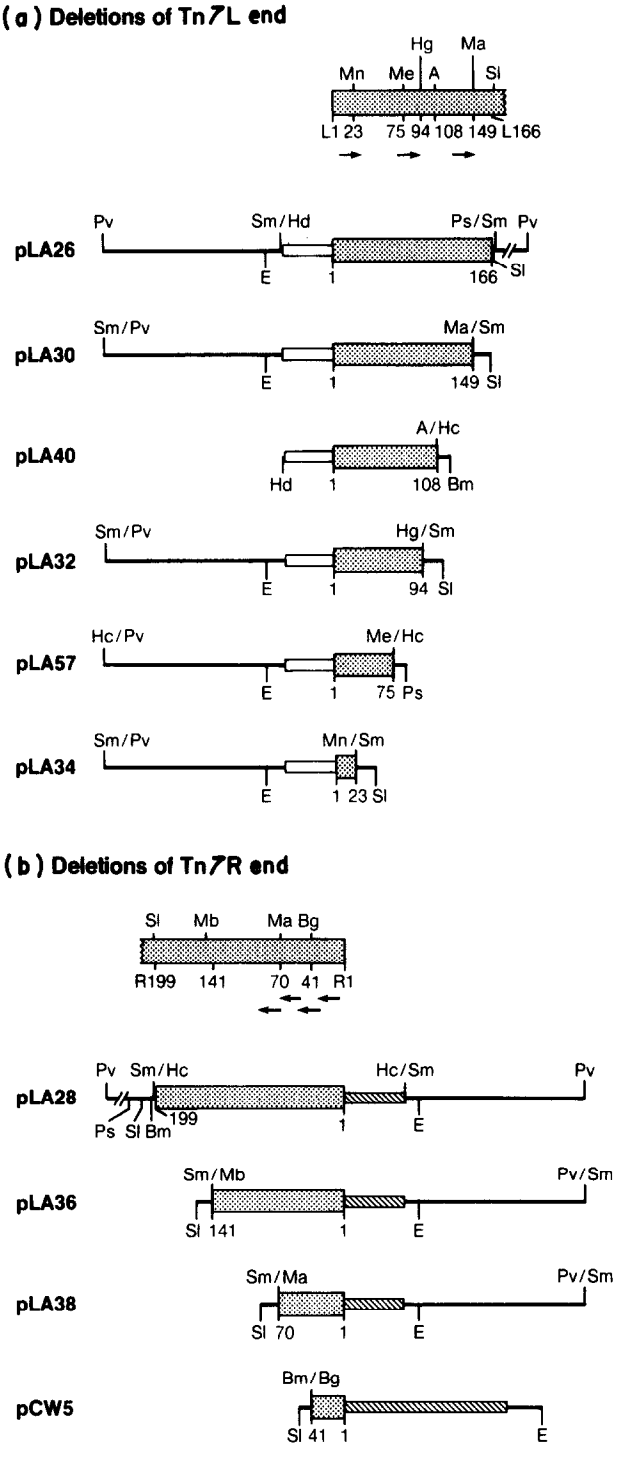

Figure 2. Deletion variants of Tn7 ends. (a) Tn7L ends. (b) Tn7R ends. The Tn7 end segments are shown at the top of each panel by stippled boxes and the end sequences are numbered. The positions of restriction enzyme sites used in the construction of deletion variants are indicated and the positions of the 22 bp sequence repeated within each Tn7 end is shown (arrows; see the text). The bottom sections of each panel show portions of plasmids which contain Tn7 end deletion variants; their construction is described in Materials and Methods. The Tn7 end segments are indicated by stippled boxes, the chromosomal sequences that lie to the left of the specific insertion point in *attTn7* by open boxes, chromosomal sequences that lie to the right of the specific insertion point in *attTn7* by hatched boxes and vector sequences by lines. Restriction sites used in the construction of these plasmids are indicated on the top of the plasmid segments and restriction enzyme sites used in the assembly of miniTn7 elements from these segments on the bottom of the plasmid segments as described in Materials and Methods. Restriction enzyme sites; A, *Alu*I; Bg, *Bgl*II; Bm, *Bam*HI; E, *Eco*RI; Hc, *Hin*cII; Hd, *Hin*dIII; Hg, *Hga*I; Ma, *Mae*I; Mb, *Mbo*II; Me, *Mae*III; Mn, *Mnl*I; Ps, *Pst*I; Pv, *Pvu*II; Sl, *Sal*I; and Sm, *Sma*I.

terminus by *E. coli* chromosomal sequences that lie to the right (towards *glmS*) of the specific insertion point in *attTn7* (Tn7R/*attTn7*+64). It was obtained by insertion of the Tn7R-containing *Hin*cII fragment from pLA1 into the *Sma*I site of pUC18 with *attTn7* sequences adjacent to vector *plac*.

pLA36 (R141) and pLA38 (R70) contain shorter Tn7R segments obtained by restriction enzyme digestion. The Tn7R-containing *Pvu*II fragment from pLA28 was isolated by electroelution, digested with *Mbo*II or *Mae*I, the Tn7R/*attTn7* fragments were treated with Klenow fragment and inserted into *Sma*I site of pUC18 with *attTn7* sequences adjacent to vector *plac*.

pCW5 contains Tn7R41/*attTn7*+165. It was constructed by isolation of the Tn7R-containing *Bgl*II–*Eco*RI fragment from pRM2::Tn7 and insertion of this fragment into the *Bam*HI–*Eco*RI site of pUC18 (C. Waddell & N. Craig, unpublished results).

(vi) *Construction of miniTn7 element plasmids*

All miniTn7 elements contain a Km^R gene, except miniTn7*L1900* × *R537*, which contains a Cm^R gene. Plasmids containing miniTn7 elements are listed in Table 1. pRM2::miniTn7*L1900* × *R537* and pLA1 are described above. All other plasmids containing miniTn7 elements, except pDD3 and pLA5, were assembled by simultaneous ligation of fragments containing: (1) Tn7 end segments (either a Tn7L and a Tn7R segment, 2 Tn7L segments or 2 Tn7R segments), (2) a Km^R segment (from pUC-4K) and (3) a vector backbone segment (either pUC18 or pACYC184). Tn7L166 fragments were obtained from either pLA26 or pLA1; fragments containing shorter Tn7L end segments were obtained from the plasmids shown in Fig. 2(a). Tn7R199 fragments were obtained from either pLA28 or pLA1; fragments containing shorter Tn7R segments were obtained from the plasmids shown in Fig. 2(b). The Tn7 end restriction fragments used were dictated by the Km^R and vector fragments used. The Km^R fragment used had terminal *Sal*I sites except for pLA47, pLA66 and pLA5, where it had terminal *Bam*HI sites and pLA59, where it had terminal *Pst*I sites. The vector cloning sites used are shown in Table 1.

Several examples of miniTn7 element plasmid constructions are given below. pLA48, which contains miniTn7*L94* × *R199*, was constructed by simultaneous ligation of the *Eco*RI–*Sal*I Tn7L94 fragment from pLA32, the *Sal*I–*Sal*I Km^R fragment from pUC-4K, the *Sal*I–*Eco*RI Tn7R199 fragment from pLA1 and *Eco*RI digested pUC18 and selection for Km^R. pLA47, which contains miniTn7*L108* × *R199*, was constructed by simultaneous ligation of the *Hin*dIII–*Bam*HI Tn7L108 fragment of pLA40, the *Bam*HI–*Bam*HI Km^R fragment from pUC-4K, the *Bam*HI–*Eco*RI fragment from pLA28 and *Hin*dIII–*Eco*RI-digested pUC18 and selection for Km^R. The orientations of the miniTn7 elements and the

Table 1
Plasmids carrying miniTn7 elements

Plasmid†	miniTn7 elements and flanking sequences‡	Vector and cloning sites used§
pRM2::mini-Tn7*L1900* × *R537*	−342/*L1900* × *R537*/*att*+165	pUC18
pLA1	−342/*L166* × *R199*/*att*+165	pUC18
pLA43	−52/*L149* × *R199*/*att*+165	pUC18-*Eco*RI
pLA47	−52/*L108* × *R199*/*att*+64	pUC18-*Hin*dIII,*Eco*RI
pLA48	−52/*L94* × *R199*/*att*+165	pUC18-*Eco*RI
pLA59	−52/*L75* × *R199*/*att*+64	pUC18-*Eco*RI
pLA45	−52/*L23* × *R199*/*att*+165	pUC18-*Eco*RI
pLA49	−342/*L166* × *R141*/*att*+64	pUC18-*Hin*dIII,*Eco*RI
pLA50	−342/*L166* × *R70*/*att*+64	pUC18-*Hin*dIII,*Eco*RI
pLA13	−342/*L166* × *R41*/*att*+165	pUC18-*Hin*dIII,*Eco*RI
pLA66	*L166F*/−342 × *R199*/*att*+64	pUC18-*Eco*RI
pDD1	*att*+165/*R199* × *R199*/*att*+165	pACYC184-*Eco*RI
pDD2	−342/*L166* × *L166*/−342	pACYC184-*Eco*RI
pDD3	−342/*R199* × *R199*/*att*+165	pUC18
pLA55	*att*+64/*R70* × *R70*/*att*+64	pUC18-*Eco*RI
pLD1	*att*+165/*R41* × *R199*/*att*+165	pACYC184-*Eco*RI
pLA5	−342/*R41* × *R41*/*att*+165	pUC18

† The plasmids were constructed as described in Materials and Methods.

‡ The miniTn*7* elements contain Tn7L and Tn7R segments as indicated and a drug resistance segment. miniTn*7L1900* × *R537* provides Cm^R and all other miniTn7s provide Km^R. The miniTn*7* elements are flanked by sequences that surround the specific point of Tn*7* insertion in the *E. coli* chromosome. The numbering system for this region is described in Materials and Methods. −342/L or −52/L indicates that the terminus of the Tn7L segment at L1 is flanked by either 342 nucleotides or 52 nucleotides from the *E. coli* chromosome that lie between the point of Tn*7* insertion and *phoS*. *R*/*att*+165 or *R*/*att*+64 indicates that the terminus of the Tn7R segment at R1 is flanked by sequences that extend from the point of insertion towards *glmS*. The designation *att* is used for these sequences because they contain the sequence information necessary for *attTn7* target activity (Gringauz *et al.*, 1988).

§ As described in Materials and Methods, the indicated restriction sites in the vector backbone were used in the assembly of miniTn*7* element plasmids. When no site is indicated, plasmid is recovered in *in vivo* transposition into *attTn7*-containing plasmid.

Km^R fragment within these plasmids are not known for all plasmids.

pDD3 was constructed by *in vivo* transposition of miniTn*7R199* × *R199* into *attTn7* of pRM2. pLA5 was constructed by digestion of pDD3 with *Bgl*II and introduction of a Km^R segment with terminal *Bam*HI sites from pUC-4K.

(vii) *Plasmids containing single Tn7-end segments used in* trans-*inhibition experiments*

Plasmids containing single Tn*7*-end segments that were used in the *trans*-inhibition experiments are shown in Fig. 3.

pLA19, which contains Tn7L166, was constructed by isolating the *Hin*dIII–*Sal*I Tn*7*166 fragment from pLA1, treating it with Klenow fragment, and inserting it into the *Dra*II site of pUC18, which had been treated with Klenow fragment. with Tn7L sequences adjacent to vector p*lac*.

pLA17, which contains Tn7R199/*attTn7*+165, was constructed by isolating the *Eco*RI–*Sal*I Tn7R fragment from pLA1, treating it with Klenow fragment and inserting it into the *Dra*II site of pUC18, with Tn*7* sequences adjacent to vector p*lac*.

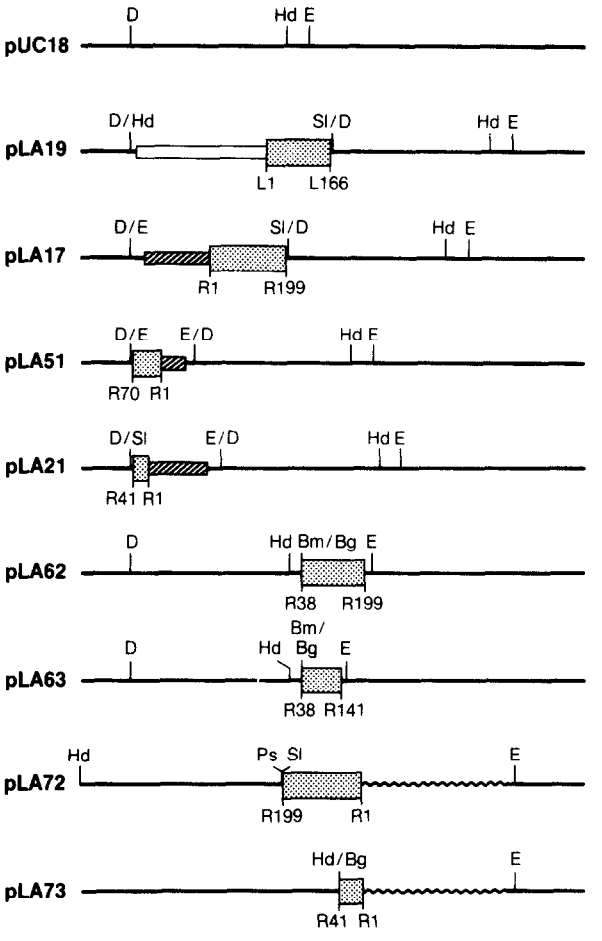

Figure 3. Plasmids containing Tn*7* end segments. The construction of these plasmids, which were used in the *trans* inhibition experiments, is described in Materials and Methods. The stippled boxes represent the Tn*7* end segments, the open boxes represent *E. coli* sequences that flank Tn7L in chromosomal *attTn7*, the hatched boxes represent chromosomal sequences that flank Tn7R in chromosomal *attTn7*, the wavy lines represent chromosomal sequences that flank Tn7R in site EC-18 and the straight lines represent vector sequences. The nucleotide positions at the boundaries of Tn7L and Tn7R segments are indicated. Restriction enzyme sites: Bg, *Bgl*II; Bm, *Bam*HI; D, *Dra*II; E, *Eco*RI;Hd, *Hin*dIII; Ps, *Pst*I; and Sl, *Sal*I.

pLA36, which contains Tn7R141/*attTn7*+64, was constructed as described above in section (d)(v) and is shown in Fig. 2(b).

pLA51, which contains Tn7R70/*attTn7*+64, was constructed by isolating the *Eco*RI Tn7R fragment from a plasmid pLA39, which is similar to pLA38, except that Tn7R70/*attTn7*+64 fragment was inserted into the vector in opposite orientation, treating it with Klenow fragment and inserting it into the *Dra*II site of pUC18 with *attTn7* sequences adjacent to vector p*lac*.

pLA21, which contains Tn7R41/*attTn7*+165, was constructed by isolating the *Sal*I–*Eco*RI Tn7R fragment from pCW5, treating it with Klenow fragment and inserting it into the *Dra*II site of pUC18 with *attTn7* sequences adjacent to vector p*lac*.

pLA62, which contains Tn7R sequences from positions R38 to position R199, is a derivative of pLA29. pLA29 is

similar to pLA28 except that the Tn7R199/*attTn7* + 64 fragment was inserted into the vector in opposite orientation. pLA62 was constructed by digestion of pLA29 with *Bam*HI and *Bgl*II and then religating the vector, thus removing the terminal Tn7R and *attTn7* sequences.

pLA63, which contains Tn7R sequences from position R38 to position R141, is a derivative of pLA37. pLA37 is similar to pLA36 except that the Tn7R/*attTn7* segment was inserted into the vector in opposite orientation. pLA63 was constructed by digestion of pLA37 with *Bam*HI and *Bgl*II and vector religation, thus removing the terminal Tn7R and *attTn7* sequences.

pLA72, which contains Tn7R199 flanked by *E. coli* chromosomal sequences unrelated to *attTn7* (Tn7R199/EC-18), was constructed by digestion of pEM-1 with *Hin*dIII and vector religation, thus removing the Tn7L sequences and part of the Km^R segment.

pLA73, which contains Tn7R41 flanked by *E. coli* chromosomal sequences unrelated to *attTn7* (Tn7R/EC-18) was constructed by digestion of pEM-1 with *Hin*dII and *Bgl*II, treatment with Klenow fragment and vector religation, thus removing the Tn7 sequences, the Km^R segment and part of the Tn7R sequences.

(viii) *Construction of pOX*-attTn7

NLC51 was lysogenized with λ 1046, which contains the Tn*10* transposase gene under control of a p*tac* promoter (Morisato *et al.*, 1983). The lysogen was then transformed with pRM4(McKown *et al.*, 1988), which contains an *attTn7* −342 to +165 segment within a Tc^R miniTn*10* element incapable of transposition in the absence of exogenous Tn*10* transposase. The F plasmid derivative lacking any known transposable element pOX38-Gen (Johnson & Reznikoff, 1984), referred here to as pOX, was then transferred into the lysogen by conjugation. pOX derivatives containing the *attTn7* miniTn*10* element resulting from Tn*10* transposition were obtained by conjugation of pOX and selection for Tc^R exconjugants. Southern analysis of transposition products into this plasmid confirmed the presence of a single *attTn7* sequence in this plasmid (Waddell & Craig, 1988).

(ix) *Construction of target plasmids containing miniTn7 elements used in transposition immunity and* attTn7 *inactivation experiments*

pOX derivatives containing various miniTn7 elements were obtained by transposition of the miniTn7 elements from donor plasmids in hosts containing chromosomal Tn7 and mating out of the episome.

pOX-*attTn7*,EP-18 :: miniTn7*L166* × *R199* (*tnsE* plasmid), pOX-*attTn7*,EP-19 :: miniTn7*L166* × *R199* and pOX-*attTn7*,EP-20 :: miniTn7*L166* × *R199* contain miniTn7*L166* × *R199* at positions other than *attTn7* Fig. 3, lines 9, 12, 11 of Waddell & Craig, 1988). They were obtained by transposition of miniTn7*L166* × *R199* from chromosomal *attTn7* to pOX-*attTn7* in a host containing pCW4 :: miniMu72, which provides *tnsABC* + *tnsE*. Physical analysis of these plasmids showed that the miniTn7 insertions were in restriction fragments other than that containing *attTn7*.

pOX-*attTn7* derivatives containing miniTn7 elements in *attTn7* (pOX-*attTn7* :: miniTn7) were obtained by transposition of the miniTn7 elements from donor plasmids in hosts containing chromosomal Tn7 and mating out of the episome. The high frequency of miniTn7 transposition to pOX-*attTn7* indicates that the elements inserted into *attTn7*.

(e) *Introduction of miniTn7 elements into chromosomal* attTn7

miniTn7*L1900* × *R537* and miniTn7*L166* × *R199* insertions into chromosomal *attTn7* were obtained as described (Waddell & Craig, 1988). Other miniTn7 insertions were obtained as follows: the miniTn7s were transposed from donor plasmids in LA3 (NLC51 *attTn7* :: Tn7) to an F plasmid derivative temperature-sensitive for replication, F'ts*lac* (Beckwith *et al.*, 1966); F'ts *lac* :: miniTn7 products were recovered by conjugation and appropriate selection. Subsequently, F'ts *lac* :: mini-Tn7s were introduced by conjugation into an NLC51 derivative containing the *tns* gene plasmid pCW4; the chromosomal *attTn7* site of this strain was vacant. Temperature exclusion of the F'*ts* episome was then carried out by growth at high temperature in liquid media containing kanamycin (miniTn7 marker) and tetracycline (pCW4 marker). Cells lacking the episome and containing chromosomal miniTn7 insertions were identified by plating on Km + Tc + 5-bromo-4-chloro-3-indolyl-β-D-galactoside plates.

(f) *Mating out assay for transposition*

In this assay, a Tn7 element transposes in a donor cell to a conjugable target plasmid and transposition is detected by identification of a Tn7 element-containing transconjugant. All mating out transposition assays were carried out in derivatives of NLC51. The mating out assay was used to measure both *tnsABC* + *tnsD* and *tnsABC* + *tnsE*-dependent transposition; pOX-*attTn7* and pOX were used as target molecules, respectively (Waddell & Craig, 1988). Transposition was measured under 2 different conditions: (1) the transposable elements were located in plasmid donor molecules and the transposition proteins were supplied by chromosomal Tn7 in *attTn7*; or (2) transposable elements were located in chromosomal *attTn7* and transposition proteins were supplied by a *tns* plasmid such as pCW4. CW51 (Rif^R) was used as the recipient strain. Assays were carried out as described in Waddell & Craig (1988). Portions of the donor cultures were also plated on media containing appropriate antibiotics to determine that the donor plasmid was present in the cells used in the mating mixtures.

With both pOX and pOX-*attTn7* targets, the total number of exconjugants was determined by selection on Gen + Rif plates. The number of exconjugants containing Tn7 or miniTn7 elements was determined by selection on Tp + Rif, Cm + Rif or Km + Rif plates, as appropriate. Transposition efficiency was calculated as the ratio of the number of Tn7 element-containing exconjugants to the total number of exconjugants. In some experiments, the mating mixtures were also plated on media containing the appropriate antibiotics to identify exconjugants containing transferred donor plasmids.

(g) *Lambda hop assay for transposition*

In this assay, a miniTn7 element transposes from a replication and integration-defective phage lambda derivative upon infection into cells containing the *tns* genes; transposition is detected by isolation of cells containing the miniTn7 element by selection on plates containing appropriate antibiotics (McKown *et al.*, 1988). Lambda KK1 was used as the donor vehicle for miniTn7*L166* × *R199*, which carries Km^R. Lambda KK1 is lambda 780 *hisG* 9424 :: Tn*10* *del*16 *del*17 :: *attTn7* −342 to +165 :: miniTn7*L166* − *KmR* − *R199* (McKown *et*

al., 1988). The recipient cells were derivatives of NLC51 containing various plasmids as described in Table 6. Assays were carried out as described by McKown *et al.* (1988).

(h) *Evaluation of site and orientation-specificity of insertion into* attTn7

To determine site and orientation-specificity of various miniTn*7* elements, we analyzed by restriction enzyme digestion the products of independent insertions of these elements into the *attTn7* plasmid pRM2. miniTn*7* insertions in pRM2 were recovered by 2 different methods. (1) Insertions of miniTn*7L166* × *R199*, miniTn*7L149* × *R199*, miniTn*7L166* × *R70* and miniTn*7R199* × *R199* into pRM2 were recovered by isolation of plasmid DNA from LA3 strains containing pOX :: miniTn*7* derivatives and pRM2 and subsequent transformation to another host selecting for Km^R, the marker carried by the miniTn*7* elements. (2) Insertions of miniTn*7R199* × *R199* and miniTn*7R41* × *R199* into pRM2 were recovered by isolation of plasmid DNA from Km^RCb^R cells lacking the F episome, obtained after growth at 42°C in liquid LB+Km+Cb of NLC51 derivatives containing Tn*7* in chromosomal *attTn7* and F'ts*lac* :: miniTn*7* elements.

3. Results

(a) *Tn7 termini contain the* cis*-acting transposition sequences*

To identify Tn*7*'s *cis*-acting transposition sequences, we analyzed the transposition properties of miniTn*7* elements containing Tn*7*L and Tn*7*R end segments of different lengths. We use the nomenclature miniTn*7L1900* × *R537* to describe a miniTn*7* element in which a fragment of the left end of Tn*7* extending from the terminus L1 to position L1900 and a fragment of the right end of Tn*7* extending from the terminal position R1 to position R537 flank a drug resistance marker (Fig. 1(b)).

Transposition of miniTn*7*s, supported by Tn*7*-encoded transposition proteins supplied *in trans*, to conjugable target plasmids was measured by mating out assays. To evaluate low-frequency, random target site transposition mediated by *tnsABC* + *tnsE*, we used as a target an F plasmid derivative, pOX38-Gen, hereinafter referred to as pOX (Johnson & Reznikoff, 1984; Waddell & Craig, 1988). To evaluate high-frequency, target site-specific transposition mediated by *tnsABC* + *tnsD*, we used as a target a pOX derivative carrying Tn*7*'s specific insertion site, *attTn7*, hereafter referred to as pOX-*attTn7* (see Material and Methods; Waddell & Craig, 1988).

We examined transposition of miniTn*7* elements under two different conditions in which the copy number of the miniTn*7* elements, and the copy number of the Tn*7* transposition (*tns*) genes varied. In one condition, miniTn*7* elements in multi-copy, non-mobilizable donor plasmids were supplied with Tns proteins from intact Tn*7* in chromosomal *attTn7*. This situation allowed analysis of both simple insertion and cointegrate transposition products. In the other condition, single-copy miniTn*7*s in chromosomal *attTn7* were supplied with Tns proteins from a *tns* plasmid. This situation minimized differences in sequences flanking the miniTn*7*s and possible differences in both copy number of the miniTn*7* donor plasmids and *tns* gene expression mediated by regulatory sites within the transposon end segments (see below).

Intact Tn*7* transposes to pOX at a low frequency ($1{\cdot}7 \times 10^{-4}$ transposon-containing transconjugants/transconjugant) and at a much higher frequency ($9{\cdot}4 \times 10^{-3}$) to pOX-*attTn7*, reflecting the high efficiency of site-specific insertion in *attTn7*. Work by Hauer & Shapiro (1984) indicated that miniTn*7L1900* × *R537* contains Tn*7*'s essential *cis*-acting transposition sequences. Our analyses of multi-copy (Table 2A) and single-copy (Table 2B) miniTn*7L1900* × *R537* transposition support this view. Under either condition, miniTn*7L1900-R537* transposes at low frequency to pOX and much higher frequency to pOX-*attTn7*. It should be noted that the same total number of transposition events was observed with either multi-copy or single-copy miniTn*7L1900-R537*. Thus, the transposition frequency per miniTn*7* element was much lower with the multi-copy miniTn*7* elements. The failure to observe an increase in total transposition frequency when the number of transposition substrates was increased could mean that the transposition frequency was limited by the concentration of a transposition protein. However, the actual concentrations of transposition proteins are unknown under either the single-copy or multi-copy miniTn*7* conditions.

(b) cis*-acting transposition sequences are contained within the L166 and R199 segments*

We evaluated the transposition properties of miniTn*7L166* × *R199*, an element containing short Tn*7* end segments. We found that this element transposes as efficiently as miniTn*7L1900* × *R537* to both pOX and pOX-*attTn7* (Table 2A,B). These two elements have also been shown to transpose at equal frequency to *attTn7* using a different transposition assay (McKown *et al.*, 1988). Like intact Tn*7*, miniTn*7L166* × *R199* inserts into *attTn7* in site and orientation-specific fashion, as demonstrated by restriction enzyme analysis of a number of independent insertions into the *attTn7* plasmid pRM2 (obtained as described in Materials and Methods; data not shown) and by nucleotide sequences analysis of *attTn7* :: miniTn*7L166* × *R199* insertions (McKown *et al.*, 1988). Thus the L166 and R199 segments contain the *cis*-acting sequences that are required for efficient Tn*7* transposition.

To determine whether miniTn*7L166* × *R199* transposition products are simple insertions or cointegrates, we examined the transfer of the donor plasmid backbone by screening miniTn*7*-containing transconjugants (as in Table 2A, line 3) for the presence of the donor plasmid backbone marker

Table 2

Transposition of mini-Tn7s containing short Tn7 end segments

Tn7 or miniTn7 element	Transposition proteins	Transposition frequency† A. *From multi-copy plasmids*‡ pOX	pOX-*attTn7*	B. *From the chromosome*§ pOX	pOX-*attTn7*
Tn7	+	nt	nt	$4{\cdot}5(\pm 0{\cdot}9)\times 10^{-5}$ (9)	nt
L1900 × *R537*	+	$8{\cdot}9(\pm 2{\cdot}1)\times 10^{-5}$ (3)	$7{\cdot}3(\pm 1{\cdot}9)\times 10^{-2}$ (3)	$9{\cdot}1(\pm 1{\cdot}7)\times 10^{-5}$ (9)	$4{\cdot}0(\pm 0{\cdot}9)\times 10^{-2}$ (9)
L166 × *R199*	+	$9{\cdot}8(\pm 1{\cdot}6)\times 10^{-5}$ (12)	$6{\cdot}1(\pm 4{\cdot}1)\times 10^{-2}$ (6)	$4{\cdot}6(\pm 0{\cdot}7)\times 10^{-4}$ (5)	$5{\cdot}7(\pm 1{\cdot}7)\times 10^{-2}$ (5)
L166 × *R199*	−	$<1{\cdot}3(\pm 0{\cdot}3)\times 10^{-7}$ (3)	$2{\cdot}8(\pm 0{\cdot}6)\times 10^{-6}$ (10)‖	$<1{\cdot}6(\pm 0{\cdot}03)\times 10^{-7}$ (3)	$<7{\cdot}2(\pm 0{\cdot}9)\times 10^{-9}$ (4)
L166F × *R199*	+	$<9{\cdot}4(\pm 0{\cdot}8)\times 10^{-8}$ (3)	$8{\cdot}5(\pm 0{\cdot}3)\times 10^{-6}$ (3)¶	nt	nt

† Transposition to the indicated target plasmids was evaluated by mating out assays as described in Materials and Methods. The transposition values are the means ±s.e.m. (number of trials is given in parentheses) and were obtained by determining the ratio of the element containing transconjugants (Tp^RRif^R, Km^RRif^R or Cm^RRif^R) to the total number of transconjugants (Gm^RRif^R).

‡ Tns proteins were provided in the indicated experiments by intact Tn7 in chromosomal *attTn7*. The donor plasmids were pRM2 :: miniTn7*L1900* × *R537*, pLA1 for miniTn7*L166* × *R199* and pLA66 for miniTn7*L166F* × *R199*.

§ Tns proteins were provided in the indicated experiments (including experiments with Tn7) by pCW4 except for miniTn7*L1900* × *R537* to pOX-*attTn7*, which was supported by pCW4 derivatives containing miniMu insertions in regions non-essential for Tn7 transposition (Waddell & Craig, 1988). The values for Tn7 transposition, miniTn7*L1900* × *R537* transposition to pOX-*attTn7*, and miniTn7*L166* × *R199* transposition to pOX-*attTn7* in the absence of Tns proteins were kindly provided by C. Waddell (Waddell & Craig, 1988).

‖ All Km^R transconjugants in the indicated experiments contained the donor plasmid, i.e. they were $Km^RCb^RRif^R$. As described in the text, they likely contain hybrid plasmids generated by a homologous recombination-driven single crossover between *attTn7* sequences in the donor and target plasmids. Km^R Cb^S Rif^R transconjugants that could result from a double crossover have never been observed (detectable frequency $<3{\cdot}5\times 10^{-7}$). The hybrid plasmids could be transferred to another host by conjugation and were stable in a Tn7-containing host.

¶ A very few Km^R Cb^S transconjugants (frequency $6{\cdot}6\times 10^{-7}$) were recovered among the Km^R Cb^R products. It is unclear whether they are the products of a very inefficient transposition reaction utilizing the inverted Tn7L end (or some other sequence in the donor plasmid) or result from transposition-independent processes.

Cb^R. No donor plasmid transfer, i.e. no cointegrate formation, was observed (detectable frequency $<1\%$ of the transposition products). Thus, formation of simple insertions is the predominant reaction in both the *tnsD* and *tnsE*-dependent transposition pathways.

Transposition of miniTn7*L166* × *R199* requires the Tns proteins (Table 2, line 4). We suggest that the very low level transfer of this element observed in the absence of the Tns proteins with the pOX-*attTn7* target (Table 2A, line 4) likely reflects a homologous recombination-driven single crossover between the donor and target plasmids; all such transconjugants contained the donor plasmid backbone marker Cb^R in addition to the miniTn7 marker Km^R. Such recombination could occur between *attTn7* sequences in the target pOX-*attTn7* plasmid and the *attTn7* sequences flanking the miniTn7 element in the donor plasmid. Low-level transfer of the donor plasmid backbone was also observed with a transposition-defective miniTn7 element (Table 2, line 5). Our experiments were performed in *recA*$^-$ strains; however, *recA*-independent recombination involving plasmids has been observed (O'Connor *et al.*, 1986; Cohen *et al.*, 1985). *tns*-independent transfer of miniTn7*L166* × *R199* without concommitant transfer of the donor plasmid backbone marker, which could result from a double crossover on both sides of the miniTn7 has not been observed (detectable frequency of Km^RCb^S products $<3{\cdot}5\times 10^{-7}$). We also examined the orientation requirement of the Tn7 ends by analyzing the transposition of miniTn7*L166F* × *R199*, an element in which L166 has been inverted with respect to its natural orientation in Tn7. No transposition of this element to pOX was detected (Table 2, line 5).

We conclude that sequences that participate directly in Tn7 transposition in both the *tnsABC* + *tnsD* and the *tnsABC* + *tnsE* pathways are contained within the L166 and R199 terminal segments and that a specific orientation of these segments is required for efficient transposition. The products of miniTn7*L166* × *R199* transposition are simple insertions.

(c) *Identification of transposition sequences within Tn7L166 and Tn7R199*

To localize further the *cis*-acting transposition sequences within the Tn7 end segments of miniTn7*L166* × *R199*, we constructed deletions of both end segments extending from their internal boundaries towards their termini and analyzed the transposition properties of miniTn7 elements containing various L ends and R199, and of elements containing L166 and various R ends. Similar results were obtained from analysis of both multi-copy and single-copy miniTn7s containing deleted end segments (Table 3). These experiments showed that extensive sequence information in both Tn7L and Tn7R is required for transposition. Furthermore, Tn7L and Tn7R segments of different lengths are required for transposition.

Table 3
Identification of Tn7's cis*-acting transposition sequences*

miniTn7 element	Transposition frequency (%)†							
	A. *From multi-copy plasmids*‡				B. *From the chromosome*			
	pOX		pOX-*attTn7*		pOX		pOX-*attTn7*	
L166 × *R199*	100	(12)	100	(6)	100	(5)	100	(5)
L149 × *R199*	530	(4)	54	(5)	176	(5)	333	(3)
L108 × *R199*	≤0·3	(4)	0·1	(5)	1·1	(5)	1·0	(5)
L94 × *R199*	1·0	(4)	0·1	(4)	1·8	(4)	1·6	(5)
L75 × *R199*	≤0·5	(5)	0·1	(5)	0·7	(5)	nt	
L23 × *R199*	<0·1	(3)	<0·0002	(3)	nt		nt	
L166 × *R141*	184	(5)	102	(5)	141	(4)	nt	
L166 × *R70*	27	(5)	18	(5)	26	(5)	51	(5)
L166 × *R41*	<0·09	(3)	0·02	(5)	nt		nt	

† Transposition was evaluated by mating out assays as described in Materials and Methods and in the notes to Table 2. The transposition frequencies are expressed as a percentage of miniTn*7L166* × *R199* transposition from a multi-copy plasmid and from the chromosome as given in Table 2, line 3. The number of trials is given in parentheses.

‡ The donor plasmids used in these experiments were: line 1, pLA1; 2, pLA43; 3, pLA47; 4, pLA48; 5, pLA59; 6, pLA45; 7, pLA49; 8, pLA50; and 9, pLA13. Km^R transconjugants were tested for the presence of the donor plasmid backbone marker Cb^R. No Km^RCb^R transconjugants were recovered in experiments when pOX was a target, or when pOX-*attTn7* was the target with miniTn7 elements that transposed efficiently (lines 1, 2, 7, 8). When pOX-*attTn7* was the target for elements that transposed poorly (lines 3 to 6 and 9), Km^RCb^R transconjugants were recovered at a frequency roughly the same as observed in the absence of Tns proteins (Table 2, line 4). Only Km^RCb^S transconjugants were considered in determining the transposition frequency of these miniTn7 elements.

Analysis of miniTn7s containing various Tn7L segments revealed that miniTn*7L149* × *R199* transposed efficiently to both pOX and pOX-*attTn7* (line 2). However, transposition of miniTn7s containing L108 or smaller L end segments was markedly reduced (at least 100-fold; lines 3 to 5). Thus, although sequences within the region between positions L1 and L75 can support infrequent transposition, sequences within the region between positions L108 and L149 are required for efficient transposition. Analysis of elements containing deleted Tn7R ends revealed that whereas miniTn*7L166* × *R141* and miniTn*7L166* × *R70* transposed efficiently (lines 7, 8), transposition of miniTn*7L166* × *R41* was significantly decreased (more than 1000-fold, line 9). Thus, some sequences necessary for efficient transposition are located between positions R41 and R70. Although the end segment extending from the terminus R1 to position R41 could not support transposition in these experiments (as in miniTn*7L166* × *R41*), a result in good agreement with other observations (Smith & Jones, 1984; Ouartsi *et al.*, 1985), it contains sequences that do participate in transposition (see below). We note that although miniTn*7L166* × *R70* is highly active, its transposition frequency was slightly but consistently lower than that of miniTn7s containing longer Tn7R segments. This result, taken together with the results of other experiments described below, may indicate that, although sequences in Tn7R extending beyond position R70 are not essential for efficient transposition under our experimental conditions, they may participate in and influence transposition.

Restriction enzyme analysis of eight independent insertions of miniTn*7L149* × *R199* and miniTn*7L166* × *R70* into *attTn7* of pRM2 (data not shown) revealed that these elements insert site and orientation-specifically into *attTn7*. We also determined that the transposition products of these miniTn7s were predominantly simple insertions by examining transconjugants containing these elements for the presence of the donor plasmid backbone (data not shown). Therefore, transposition of these two miniTn7s has all the characteristics of wild-type Tn7 transposition.

The above experiments demonstrate that the *cis*-acting transposition sequences in Tn7L and Tn7R required for efficient transposition are different in length, about 150 bp in Tn7L and about 70 bp in Tn7R. Because the end deletions similarly affect transposition to pOX and to pOX-*attTn7*, the same sequences in the Tn7 ends are likely necessary for both *tnsD* and *tnsE*-dependent transposition.

(d) *Transposition of miniTn7s containing symmetrical ends*

We also analyzed transposition properties of miniTn7s containing symmetrical ends, i.e. two Tn7L segments or two Tn7R segments (Table 4). These experiments revealed that sequences in Tn7R can substitute for the *cis*-acting transposition sequences in Tn7L but that Tn7L sequences cannot substitute for Tn7R.

We were unable to detect transposition of miniTn*7L166* × *L166* to either pOX or to pOX-*attTn7* (Table 4, line 2). By contrast, we observed efficient transposition of miniTn*7R199* × *R199* and miniTn*7R70* × *R70* to both of these target plasmids (lines 3, 4). In fact, the transposition frequencies of single-copy miniTn*7R199* × *R199* and miniTn*7R70* × *R70* were close to that of miniTn*7L166* × *R199*. Transposition of miniTn*7R199* × *R199* required the same *tns* genes

Table 4

Transposition of miniTn7 elements containing two Tn7L ends or two Tn7R ends

miniTn7 element	Transposition frequency (%)†							
	A. *From multi-copy plasmids*‡				B. *From the chromosome*			
	pOX		pOX-*attTn7*		pOX		pOX-*attTn7*	
L166 × *R199*	100	(12)	100	(6)	100	(5)	100	(5)
L166 × *L166*	<0·2	(6)	<0·0005	(3)	nt		nt	
R199 × *R199*	57	(3)	64	(3)	59	(5)	77	(5)
R70 × *R70*	270	(7)	20	(4)	19	(5)	35	(5)
R41 × *R199*	3·4	(5)	0·2	(5)	1·2	(5)	1·5	(5)
R41 × *R41*	<0·09	(5)	0·14	(4)	nt		nt	

† Transposition was evaluated by mating out assays as described in Materials and Methods and in the notes to Table 2. The transposition frequencies are expressed as a percentage of miniTn7*L166* × *R199* transposition from a multi-copy plasmid or from the chromosome as given in Table 2, line 3. The number of trials is given in parenthesis.

‡ The donor plasmids used in these experiments were: line 1, pLA1, 2, pDD2; 3, pDD1; 4, pLA55; 5, pLD1; and 6, pLA5. It should be noted that pLA1, pLA55 and pLA5 are pUC18 derivatives, while pDD1, pDD2 and pLD1 are pACYC184 derivatives. It should also be noted that the flanking sequences of miniTn7*L166* × *L166* in pDD2, miniTn7*R199* × *R119* in pDD1 and miniTn7*R70* × *R70* in pLA55 are different from those of the other miniTn7 elements (see Table 1). Km^R transconjugants were tested for the presence of a donor plasmid backbone marker. In the experiments of lines 1 to 3 and 5, no transconjugants containing the donor plasmid backbone were observed (detectable frequency less than 1%). In experiments in line 4 when pOX was a target, transconjugants containing the donor plasmid marker (Cb^R) were recovered at low frequency, usually about 5%. The values in the Table represent both Km^RCb^R and Km^RCb^S transconjugants. The production of these Cb^R transconjugants is considered in the text. The transconjugant Cb^R plasmids could be transferred to another strain by conjugation and were stable in a Tn7-containing host.

as did transposition of miniTn7*L166* × *R199* (N. Craig, unpublished results). We also found that miniTn7*R41* × *R199* can transpose, albeit inefficiently (line 5). Thus the segment of Tn7R extending from positions R1 to R41 contains sequences that do participate in transposition. All miniTn7*R* × *R*s transposed at higher frequency to pOX-*attTn7* than to pOX, suggesting that they have the capacity to recognize *attTn7*. Furthermore, restriction enzyme analysis of 22 independent miniTn7*R199* × *R199* insertions and 11 independent miniTn7*R41* × *R199* insertions into the *attTn7* plasmid pRM2 (obtained as described in Materials and Methods) demonstrated that these elements insert site-specifically in *attTn7* (data not shown)†.

These experiments revealed that the *cis*-acting transposition sequences in Tn7L and Tn7R are functionally different. Other analyses of the Tn7 ends described below support this conclusion.

As described above, the products of miniTn7*L166* × *R199* transposition to pOX were simple insertions, i.e. donor plasmid backbone sequences were not present in transconjugants containing the miniTn7 element. By contrast, we found that a small fraction (usually about 5%) of miniTn7*R70* × *R70* (Table 4A, line 4) and miniTn7*R199* × *R199* transposition products from pUC-derived plasmids contained the donor plasmid backbone marker Cb^R (data not shown). Such products could result from cointegrate formation during transposition of these elements. Alternatively, transconjugants containing the donor plasmid backbone could be generated by simple insertion transposition from a dimeric donor plasmid (Berg, 1983). In this case, the transposing segment would be the donor plasmid backbone flanked by two copies of the miniTn7 element. We favor the latter view as dimeric pUC-based donor plasmids were present when backbone-containing transconjugants were recovered (data not shown) and because we did not detect donor backbone-containing transconjugants when these miniTn7s transposed from other donor plasmids (as in Table 4, line 3; data not shown).

(e) trans *inibition by multi-copy Tn7 ends*

In many of the experiments presented above, we analyzed the transposition of miniTn7s from multi-copy plasmids in the presence of single-copy

† We also examined the orientation specificity of miniTn7*R41* × *R199* and miniTn7*R199* × *R199* insertion into *attTn7*. Physical analysis of 11 independent pRM2 :: miniTn7*R41* × *R199* insertions, obtained from a single F'ts :: miniTn7*R41* × *R199* donor as described in Materials and Methods, revealed that this miniTn7 element inserts into *attTn7* with its R199 end in the same orientation as does Tn7 and miniTn7*L166* × *R199*. We were surprised to find that the symmetrical element miniTn7*R199* × *R199* inserted into *attTn7* in a single orientation (as defined by this element's internal drug resistance marker) when 8 independent pRM2 :: miniTn7*R199* × *R199* insertions, obtained from a single F'ts :: miniTn7*R199* × *R199* donor, were examined. However, we recovered both orientations of miniTn7*R199* × *R199* in *attTn7* when 14 independent pRM2 insertions of this element from several different pOX :: miniTn7*R199* × *R199* donors were examined. It remains to be determined if the observed differences in miniTn7*R199* × *R199* orientation specificity reflect some influence of sequences flanking the Tn7 termini in the donor site or the different procedures used to recover the pRM2 insertions. Further experiments are required to define the determinants of Tn7 orientation specificity.

Table 5
Tn7 transposition in the presence of trans *multi-copy miniTn7 elements*

miniTn7 element	Location	Tn7 transposition frequency (%)† pOX		pOX-*attT7*	
A. *Multi-copy*‡	Plasmid				
—		100	(9)	100	(4)
	pUC18	28	(5)	nt	
	pRM2	41	(6)	66	(5)
L166 × *R199*	pLA1	0·1	(8)	3	(3)
L166 × *L166*	pDD2	32	(3)	26	(3)
R41 × *R41*	pLA5	12	(5)	72	(4)
R199 × *R199*	pDD3	≤0·1§	(3)	nt	
R199 × *R199*	pDD1	1·8	(3)	3·9	(4)
B. *Single-copy*‖	Chromosome				
L166 × *R199*	Site no. 11	33	(4)	29	(5)
L166 × *R199*	Site no. 20	16	(5)	28	(3)

† Tn7 transposition was evaluated by mating out assays as described in Materials and Methods by determining the fraction of Tn7-containing transconjugants (Tp^RRif^R) among the total transconjugants (Gen^RRif^R). Transposition frequency is expressed as percentage of Tn7 transposition observed in the absence of an additional multi or single-copy miniTn7 element; to pOX, 100% is equal to $1{\cdot}7 \times 10^{-4}$ and to pOX-*attTn7*, 100% is equal to $9{\cdot}4 \times 10^{-3}$. The number of trials is given in parentheses.

‡ Plasmids were derivatives of pUC18, except pDD1 and pDD2, which were pACYC184 derivatives. The transposition activity of miniTn7 elements was assayed simultaneously in these experiments. MiniTn7*L166* × *R199* and miniTn7*R199* × *R199* transposed efficiently (see Table 2A, line 3, and Table 4A, line 3, respectively). miniTn7*L166* × *L166* (Table 4A, line 2) and miniTn7*R41* × *R41* (Table 4A, line 6) were transposition-defective.

§ The more effective inhibition provided by pDD3, a pUC-derived plasmid, compared to pDD1, a pACYC-derived plasmid, probably reflects differences in copy number between these plasmids.

‖ In these experiments, Tn7 was located in chromosomal *attTn7* and miniTn7*L166* × *R199* was located at other chromosomal sites. The miniTn7*L166* × *R199* insertions were likely derived by *tnsE*-dependent transposition; their construction will be described elsewhere (K. Kubo and N.L.C.). The transposition frequency of the miniTn7*L166* × *R199* elements from the chromosome was also measured in these experiments and were: from no. 11, to pOX = $3{\cdot}5 \times 10^{-5}$ and to pOX-*attTn7* = $1{\cdot}6 \times 10^{-2}$, and from no. 20, to pOX = $1{\cdot}0 \times 10^{-4}$ and to pOX-*attTn7* = $6{\cdot}7 \times 10^{-2}$.

chromosomal Tn7. We have also examined Tn7 movement under these conditions (Table 5). The presence of multi-copy miniTn7*L166* × *R199 in trans* to both Tn7 and the target plasmid dramatically reduced the frequency of Tn7 transposition in both the *tnsABC* + *tnsE* and the *tnsABC* + *tnsD* pathways (Table 5, line 4). Because little decrease in Tn7 transposition was observed in the presence of multi-copy plasmids lacking the Tn7 ends (lines 2 and 3) or in the presence of single-copy chromosomal miniTn7*L166* × *R199* (lines 9, 10), we conclude that this inhibition is mediated by the multi-copy Tn7 ends. We also examined single-copy Tn7 transposition in the presence of multi-copy miniTn7s containing two Tn7L ends or two Tn7R ends. While little inhibition by multi-copy miniTn7*L166* × *L166* (line 5) or miniTn7*R41* × *R41* (line 6) was observed, multi-copy miniTn7*R199* × *R199* was a potent *trans* inhibitor of single-copy Tn7 transposition (lines 7, 8). Thus, the presence of Tn7R segments containing the sequences in this end required for effiicient transposition is required for *trans* inhibition. Other experiments described below suggest that the transposition sequences within the R199 segment mediate this multi-copy, *trans* inhibition of Tn7 transposition.

We emphasize that, although little transposition of single-copy Tn7 was observed in the presence of multi-copy miniTn7*L166* × *R199* or miniTn7*R199* × *R199*, the miniTn7s did transpose. Indeed, the transposition frequency of multi-copy miniTn7*L166* × *R199* (Table 2A, line 3) or miniTn7*R199* × *R199* (Table 4A, line 3) was comparable to the frequency of single copy Tn7 transposition in the absence of these multi-copy miniTn7s (see also note ‡ to Table 5). One interpretation of these results is that under these specific conditions the transposition proteins are limiting, so that the apparent inhibition of Tn7 transposition by the multi-copy miniTn7s actually reflects a competition for the available transposition protein(s) between the multi-copy miniTn7s and single-copy Tn7. It should also be noted that the level of transposition protein(s) could be different in the presence of the multi-copy miniTn7 (see Discussion).

In the above experiments, we analyzed *trans* inhibition by multi-copy Tn7 ends within miniTn7 elements, which could themselves transpose. We also examined the capacity of Tn7 ends to provide *trans* inhibition individually, i.e. when not contained within a miniTn7 element. In these experiments, we used a different transposition assay in which a miniTn7 transposed from a phage lambda derivative upon infection into cells containing a *tns* plasmid and vacant chromosomal *attTn7*. Transposition was measured in the presence and absence of an additional multi-copy plasmid containing either Tn7L or Tn7R end segments (Table 6). In these experiments, as in those described above, the multi-copy Tn7 end segments were *trans* to both the transposing miniTn7 element and to the target replicon. The presence of multi-copy R199 dramatically (about 200-fold) inhibited transposition of the miniTn7 from the infecting phage (Table 6, lines 2 and 4). However, *trans*-position was unaffected by the presence of multi-copy L166 or by multi-copy R41 (lines 3, 5, 6). Thus, the multi-copy R199 segment need not be part of a miniTn7 element to provide *trans* inhibition. We also found that the capacity of multi-copy R199 to provide *trans* inhibition was independent of the sequences flanking this segment: *trans* inhibition was observed with R199 segments flanked either by *attTn7* or by other sequences unrelated to *attTn7* (compare lines 2 and 4).

What sequences within the R199 segment mediate *trans* inhibition? As multi-copy R70 can provide *trans* inhibition (Table 6, line 8), the

Table 6
Inhibition by individual multi-copy Tn7 ends

Tn7-end segment/ flanking sequence	Plasmid†	Transposition frequency (%)‡	
—	pUC18	100	(8)
R199-R1/*attTn7* + 165	pLA17	0·5	(7)
R41-R1/*attTn7* + 165	pLA21	120	(5)
R199-R1/EC-18	pLA72	1·0	(9)
R41-R1/EC-18	pLA73	186	(9)
−345/L1-L166	pLA19	110	(3)
R141-R1/*attTn7* + 64	pLA36	1·1	(4)
R70-R1/*attTn7* + 64	pLA51	1·5	(4)
R38-R199	pLA62	85	(4)
R38-R141	pLA63	83	(4)

† The plasmids containing the Tn7 end segments were pUC18 derivatives obtained as described in Materials and Methods. Tn7 end segments were flanked either by *attTn7* sequences or by sequences unrelated to *attTn7* as indicated. Similar results were also obtained with plasmids containing the end segments in different sites in the vector backbone and/or in orientations different from those used in the experiments shown in lines 2, 3, 7, 8 and 10 (data not shown).

‡ Transposition was evaluated by lambda hop assays performed as described in Materials and Methods. Transposition of miniTn*7L166* × *R199* from lambda KK1 was supported by transposition proteins from plasmid pCW4 (*tnsABCDE*) except lines 4 and 5 where pCW4 :: miniMu78 (*tnsABCD*) was used. The frequency of transposition is expressed as a percentage of miniTn*7L166* × *R199* transposition in the presence of pUC18. For hosts containing pCW4, 100% is equal to $9{\cdot}2 \times 10^{-5}$ transpositions/plaque forming unit and for hosts containing pCW4 :: miniMu78, 100% is equal $3{\cdot}7 \times 10^{-4}$ transpositions/plaque forming unit. The number of trials is given in parentheses.

Table 7
Transposition immunity in the tnsABC + tnsE *pathway*

Target plasmid	Tn7 transposition frequency (%)†	
pOX	100	(9)
pOX :: miniTn*7L166* × *R199*	1·1	(6)‡
pOX :: miniTn*7R199* × *R199*	0·2	(3)
pOX :: miniTn*7R70* × *R70*	0·5	(9)
pOX :: miniTn*7L94* × *R199*	1·5	(2)
pOX :: miniTn*7R41* × *R199*	1·4	(9)
pOX :: miniTn*7R41* × *R41*	43	(7)
pOX :: miniTn*7L166* × *R70*	180	(4)
pOX :: miniTn*7L166* × *R141*	1·9	(5)

† The transposition of Tn7 from chromosomal *attTn7* to pOX and the indicated pOX derivatives was evaluated by mating out assays as described in Materials and Methods. The transposition frequency is expressed as percentage of Tn7 transposition to pOX; 100% is equal to $1{\cdot}7 \times 10^{-4}$. The number of trials is given in parentheses. We emphasize that only *tnsE*-dependent transposition to pOX occurs (Waddell & Craig, 1988).

‡ The rare pOX products containing both Tn7 and miniTn*7L166* × *R199* were stable after many generations of growth under non-selective conditions.

sequences required must lie between positions R70 and R1. Moreover, the sequences between positions R38 and R1 are required but not sufficient to provide *trans* inhibition (lines 9, 10, 3). It is notable that sequences between positions R70 and R1 are also required for transposition itself. This correlation is consistent with the view that the phenomenon of *trans* inhibition of single-copy Tn7 transposition by multi-copy Tn7R ends reflects a competition between sequences in these segments that interact with a limiting transposition protein(s).

(f) *Tn7 transposition immunity*

We presented evidence above that multi-copy Tn7R segments can inhibit transposition when present *in trans* to a target molecule. The Tn7 ends can also inhibit transposition *in cis*, i.e. when present in a target molecule. Hauer & Shapiro (1984) found that the presence of miniTn*7L1900* × *R537* in the low-copy number plasmid RP4 substantially reduced the frequency of subsequent Tn7 insertion into this replicon. The ability of transposon ends in a target replicon to inhibit subsequent transposon insertion into that replicon is called transposition immunity. Transposition immunity reflects "action at a distance" as the presence of the transposon ends in the target plasmid can inhibit subsequent insertion of another copy of the transposon into any other site in the target plasmid. We have found that Tn7 transposition immunity is active in both the *tnsABC* + *tnsE* and the *tnsABC* + *tnsD* transposition pathways. We have also found that the presence of Tn7R end segments in a target molecule is sufficient to provide immunity and that Tn7R ends of sufficient length to contain the sequences necessary for efficient transposition are required to provide immunity to a target replicon.

(i) *The* tnsABC + tnsE *pathway*

We evaluated Tn7 transposition immunity in the *tnsE*-dependent transposition pathway by measuring in mating out assays the ability of miniTn7s in the low-copy number conjugative plasmid pOX to reduce the frequency of subsequent Tn7 insertion into these plasmids. The presence of miniTn*7L166* × *R199* in pOX substantially (about 100-fold) inhibited subsequent Tn7 insertion into this target molecule (Table 7, line 2). However, the presence of single-copy miniTn7 in a replicon other than the pOX target had little effect on Tn7 transposition to pOX (Table 5, lines 9, 10). We emphasize that this inhibition is different from the *trans* inhibition provided by multi-copy Tn7 ends described above in two ways: (1) the miniTn7 element resides within the target molecule, i.e. is *cis* rather than *trans* to the target; and (2) when resident in the target molecule, single-copy miniTn7 can inhibit Tn7 transposition, whereas multi-copy miniTn7 is required for *trans* inhibition. The ability of miniTn*7L166* × *R199* in pOX to inhibit subsequent Tn7 insertion does not reflect the prior occupancy of a highly preferred Tn7 insertion site as there are no preferred Tn7 insertion sites within pOX (Waddell & Craig, 1988). Moreover, miniTn*7L166* × *R199* at several different positions in other target plasmids related to pOX all

inhibited subsequent Tn*7* insertion into these plasmids (data not shown). It is also unlikely that the low-frequency recovery of plasmids containing both Tn*7* and miniTn*7L166* × *R199* reflects the activity of a transposon-encoded resolution system as we and others (data not shown; Moore & Krishnapillai, 1982; Hassan & Brevet, 1983) have observed that plasmids containing two copies of Tn*7* are stable. Thus, the presence of Tn*7* end segments containing this element's transposition sequences in a large (greater than 50 kb) target plasmid can provide this molecule with immunity to subsequent Tn*7* insertion.

To determine which sequences within the Tn*7* ends confer transposition immunity, we examined the capacity of various miniTn*7*s in pOX to inhibit subsequent Tn*7* insertion. We found that both miniTn*7R199* × *R199* and miniTn*7R70* × *R70* could inhibit subsequent Tn*7* insertion (Table 7, lines 3, 4). Thus the sequences within R70 are sufficient whereas Tn*7*L sequences are dispensable for immunity. We also found that miniTn*7* elements incapable of efficient transposition themselves, such as miniTn*7L94* × *R199* and miniTn*7R41* × *R199* can provide immunity (lines 5, 6). It should be noted that in these miniTn*7*s, at least one end of the element does contain the Tn*7*R sequences necessary for efficient transposition. We also found that miniTn*7R41* × *R41*, both ends of which lack sequences necessary for efficient transposition could not provide immunity to a target replicon (line 7). Thus, the ability of a miniTn*7* to provide transposition immunity correlates with the presence of Tn*7*R sequences necessary for efficient Tn*7* transposition. This correlation supports the hypothesis that the same sequences at the Tn*7* ends that participate directly in transposition mediate transposition immunity when present in the target molecule.

In view of the effective transposition immunity provided by miniTn*7R70* × *R70* (Table 7, line 4), it was intriguing to find that the presence of miniTn*7L166* × *R70* in a target replicon did not reduce the frequency of subsequent Tn*7* insertion into that target, i.e. this element did not provide immunity (line 8). We interpret this result to support our speculation that some sequences that lie beyond R70, i.e. between positions R70 and R141, can contribute to both transposition immunity and, as considered above, to transposition. Perhaps the differing abilities of miniTn*7R70* × *R70* and miniTn*7L166* × *R70* to provide immunity, i.e. miniTn*7R70* × *R70* does while miniTn*7166* × *R70* does not, reflect some interaction between the Tn*7* ends resident in the target replicon that occurs more efficiently in miniTn*7R70* × *R70* than in miniTn*7L166* × *R70*.

(ii) *The* tnsABC+tnsD *pathway*

To determine whether transposition immunity is also active in the *tnsD*-dependent transposition pathway, we examined *tnsABC* + *tnsD*-promoted transposition of miniTn*7L1900* × *R537* to pOX-*attTn7* target plasmids containing miniTn*7L166* × *R199* insertions at sites other than *attTn7*. The reactivity of *attTn7* in these plasmids was markedly decreased (about 100-fold, Table 8). Thus, transposition immunity is also active in the *tnsD*-dependent transposition pathway. We note that in both the *tnsD* and *tnsE*-mediated pathways, the presence of a miniTn*7* in the target plasmid decreased the frequency of subsequent Tn*7* transposition to the same degree (about 100-fold, compare Table 7, line 2, to Table 8, lines 2 to 4).

Table 8
Transposition immunity in the tnsABC+tnsD *pathway*

Target plasmid†	Transposition frequency (%)‡	
pOX-*attTn7*	100	(3)
pOX-*attTn7*; EP-18 :: miniTn*7L166* × *R199*	0·5	(3)
pOX-*attTn7*; EP-19 :: miniTn*7L166* × *R199*	1	(3)
pOX-*attTn7*; EP-20 :: miniTn*7L166* × *R199*	1·1	(3)

† The pOX-*attTn7* plasmids containing miniTn*7L166* × *R199* were obtained by *tnsABC* + *tnsE* transposition as described in Materials and Methods; the miniTn*7* elements lie in target sites other than *attTn7* as verified by Southern analysis (Waddell & Craig, 1988).

‡ Transposition of miniTn*7L1900* × *R537* from chromosomal *attTn7* to the indicated target plasmids was evaluated by mating out assays as described in Materials and Methods. Transposition was promoted by a *tnsABCD* plasmid, pCW4 :: miniMu78. miniTn*7L1900* × *R537*-containing transconjugants were identified by $Cm^R Rif^R$ selection. Transposition frequency is expressed as the percentage of miniTn*7L1900* × *R573* transposition to pOX-*attTn7*; 100% is equal to $2{\cdot}2 \times 10^{-2}$. The number of trials is given in parentheses.

(g) *Inactivation of* attTn7 *adjacent to Tn7*

In the above experiments, we demonstrated that the presence of the Tn*7* ends in a target replicon can inhibit subsequent Tn*7* insertion when they lie at a distance from either *attTn7* or a target site unrelated to *attTn7*. We have also investigated the effects of Tn*7* ends on *attTn7* target activity when they are located in *attTn7*, i.e. in the configuration that results from Tn*7* insertion into this site. This situation was of particular interest because the nucleotides required for *attTn7* target activity lie entirely to one side of the specific point of Tn*7* insertion and do not include the insertion point itself (Gringauz *et al.*, 1988). Thus, Tn*7* insertion into *attTn7* does not physically disrupt the sequences required for *attTn7* target activity. We analyzed *tnsABC* + *tnsD*-promoted transposition of miniTn*7L1900* × *R537* to pOX-*attTn7* plasmids containing various miniTn*7* elements already resident in *attTn7*. Transposition to *attTn7* was completely blocked by a number of miniTn*7* elements including miniTn*7L166* × *R199* and various miniTn*7R* × *R* elements (Table 9). We emphasize that this inactivation of an *attTn7* adjacent to a miniTn*7* was much greater than the effects of many of these miniTn*7*s in transposition

Table 9
Inactivation of attTn7 *by adjacent Tn7 ends*

Target plasmid†	Tn7 transposition frequency (%)‡	
pOX-*attTn7*	100	(3)
pOX-*attTn7* :: miniTn7*L166* × *R199*	<0·001	(4)
pOX-*attTn7* :: miniTn7*L166* × *R141*	<0·001	(2)
pOX-*attTn7* :: miniTn7*R199* × *R199*	<0·001	(4)
pOX-*attTn7* :: miniTn7*R70* × *R70*	<0·002	(3)
pOX-*attTn7* :: miniTn7*R41* × *R199*	<0·001	(3)
pOX-*attTn7* :: miniTn7*L166* × *R70*	<0·001	(3)
pOX-*attTn7* :: miniTn7*L166* × *R41*	0·17	(3)

† The pOX-*attTn7* plasmids containing miniTn7s in *attTn7* were obtained as described in Materials and Methods.

‡ Transposition of miniTn7*L1900* × *R537* from chromosomal *attTn7* to the indicated target plasmids was evaluated by mating out assays as described in Materials and Methods. Transposition was promoted by a *tnsABCD* plasmid, pCW4 :: miniMu78. miniTn7*L1900* × *R537*-containing transconjugants were identified by $Cm^R Rif^R$ selection. Transposition frequency is expressed as a percentage of miniTn7*L1900* × *R537* transposition to pOX-*attTn7*; 100% is equal to $2{\cdot}2 \times 10^{-2}$. The number of trials is given in parentheses.

immunity. The reactivity of *attTn7* already occupied by a miniTn7 element was reduced by at least 100,000-fold whereas the reactivity of *attTn7* at a distance from a miniTn7 element was reduced only about 100-fold (compare Table 9 to Table 8). It is also notable that miniTn7*L166* × *R70*, which failed to provide detectable transposition immunity (Table 7, line 8), completely inactivated an adjacent *attTn7* (Table 9, line 7). Furthermore, even the presence of the immunity-defective miniTn7*L166* × *R41* in *attTn7* substantially inhibited subsequent insertions into this site (about 600-fold, line 8). Thus, the capacity of Tn7 ends to inactivate a nearby *attTn7* is much more pronounced than their ability to inactivate a distant *attTn7* site.

4. Discussion

The termini of transposable elements provide specific *cis*-acting sequences, recognized and acted upon by the element's transposition proteins. The Tn7-encoded transposition genes can mediate two different but related transposition reactions: (1) *tnsABC* + *tnsD* promote transposition to *attTn7* and pseudo-*attTn7* sites and (2) *tnsABC* + *tnsE* promote transposition to sites unrelated to *attTn7* (Rogers *et al.*, 1986; Waddell & Craig, 1988; K. Kubo and N. Craig, unpublished results). We have not found any differences in the transposon-carried *cis*-acting sequences required for these two reactions. Thus, it is not unreasonable to suggest that similar protein–DNA interactions at the transposon ends mediate both *tnsD* and *tnsE*-dependent transposition and that these pathways differ only in their utilization of distinct target-specific proteins, likely TnsD and TnsE, bound to their cognate DNA sites.

Extensive sequence information at each end of Tn7 is required for efficient transposition. We have shown that sequences throughout the segments extending from the left end terminus at position L1 to position L149 and from the right end terminus at position R1 to at least position R70 are likely directly involved in transposition. Thus, the *cis*-acting transposition sequences in Tn7L and Tn7R are structurally different and asymmetric. The extreme (30 bp) termini of Tn7 are highly related inverted repeats (Fig. 4; Lichtenstein & Brenner, 1982; Gosti-Testu & Brevet, 1982). However, considerably more sequence information than these repeats is required for Tn7 transposition (this work; Smith & Jones, 1984; Ouartsi *et al.*, 1985).

Although different, the ends of Tn7 are related by the presence of several highly related copies of a 22 bp sequence (Fig. 4; Lichtenstein & Brenner, 1982). Tn7L contains three directly repeated 22 bp sequences separated by unrelated sequences; Tn7R contains four 22 bp sequences arranged contiguously in direct orientation. The 22 bp sequences in Tn7L lie in inverted orientation with respect to those in Tn7R. Tn7's 30 bp terminal inverted repeats contain one copy of the 22 bp sequence and an additional 8 bp at the extreme transposon termini. Two types of evidence suggest that these 22 bp sequences are important *cis*-acting transposition sites. The 22 bp repeats are prominent features of the short Tn7L and Tn7R end segments we have found to be required for efficient transposition. The smallest Tn7R segment capable of promoting efficient transposition is R70; the majority of this segment comprises three 22 bp repeats (Fig. 4). Removal of about 1·5 of these repeats (as in R41) reduces transposition at least 1000-fold. The smallest Tn7L segment we found to

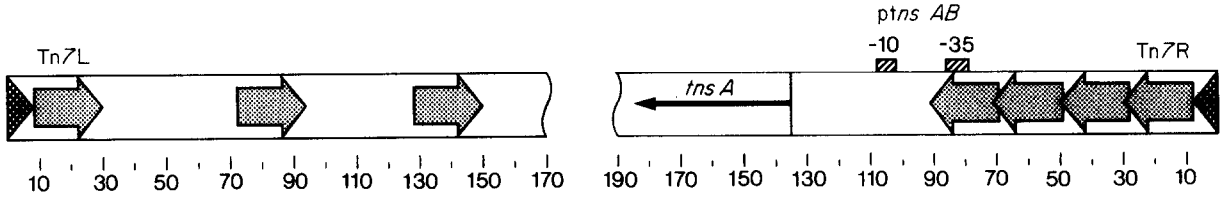

Figure 4. Structure of Tn7L and Tn7R. The Tn7L and Tn7R segments are shown and their sequences numbered. The stippled arrows represent the highly similar 22 bp repeats in each end of Tn7 (Lichtenstein & Brenner, 1982), the triangles at the extreme termini represent the 8 bp portion of Tn7's terminal inverted repeats, which are distinct from the 22 bp repeats. The proposed *tnsAB* promoter and amino-terminal coding sequence for *tnsA* are indicated (Gay *et al.*, 1984; Rogers *et al.*, 1986; Waddell & Craig, 1988).

be efficient in transposition is L149; this segment contains all three of Tn7L's 22 bp repeats. L108, which lacks one of these repeats as well as sequences between the repeats, is defective in transposition. Furthermore, other work from this laboratory has shown that TnsB can specifically recognize this sequence (McKown *et al.*, 1987; L. Arciszewska, R. McKown, N. Craig, unpublished results). It remains to be determined if other sequences in Tn7 ends, such as those which separate the 22 bp repeats in Tn7L, also have a specific role in transposition.

Although Tn7R contains four 22 bp repeats, Tn7R segments containing only three repeats are nearly as active in transposition as those with four. This finding need not contradict the hypothesis that the 22 bp repeats are important transposition signals. There are many examples of repeated protein-binding sites in which deletion of one site does not alter the apparent activity of the remaining sites (Fromm & Berg, 1983; Jayaram, 1985; Groenen *et al.*, 1985). The presence of four 22 bp repeats in Tn7R might be required under transposition conditions different than those we have used, for example when the concentrations of the proteins which act upon these repeats are lower. The 22 bp repeat sequence may also play a role in the regulation of *tns* gene expression. The proposed −35 sequence of the promoter, which directs expression of the *tnsABC* operon, is embedded within the fourth 22 bp repeat of Tn7R (Fig. 4; Gay *et al.*, 1986). Furthermore, expression from this promoter appears to be repressed in the presence of *tnsB* (Rogers *et al.*, 1986). There are known examples of other recombination sites that are also involved in the control of gene expression (Reed *et al.*, 1982; Wishart *et al.*, 1983; Wells & Grindley, 1984).

The ends of Tn7 are functionally as well as structurally asymmetric. We have demonstrated that the *cis*-acting transposition sequences in Tn7R can substitute for those in Tn7L in both *tnsD* and *tnsE*-dependent transposition, but that Tn7L cannot substitute for Tn7R. We have also found differences in the activities of the Tn7 ends in other processes related to transposition such as the ability to provide *trans* inhibition and in transposition immunity (see below). In all cases, the presence of Tn7R sequences is sufficient, while Tn7L sequences are dispensable or ineffective. These functional asymmetries of the Tn7 ends could reflect a more efficient interaction between Tn7R and a transposition protein(s) that recognizes sites common to both Tn7L and Tn7R, for example the proposed TnsB interaction with the 22 bp repeat sequences. Differential protein binding to Tn7L and Tn7R could result from different intrinsic affinities of the binding sites in each end or from different cooperative interactions between variously positioned sites within each end. Indeed, the *tnsB*-dependent end binding activity does have an apparently higher affinity for Tn7R than for Tn7L (McKown *et al.*, 1987). Alternatively, the observed differences in the activities of Tn7L and Tn7R may reflect a unique interaction of a transposition protein(s) with Tn7R.

We have found that Tn7R segments in a multicopy plasmid can inhibit the transposition of single-copy Tn7 to another target replicon. By contrast, we have not observed such *trans* inhibition by multi-copy Tn7L segments. This *trans* inhibition by multi-copy Tn7R requires the same sequences in Tn7R as are required for transposition itself. An attractive explanation is that this phenomenon actually reflects a competition for a rate-limiting transposition protein between transposition sequences in the many Tn7R segments in the multicopy plasmid and in the single copy Tn7. It should be noted that the presence of the multi-copy Tn7R segments could also affect the levels of the Tns proteins if the Tn7R segment contains a binding site(s) for a component of the *tns* regulatory circuitry. The smallest inhibitory segment (Tn7R70) lacks *tns* coding sequences or sequences complementary to the 5′ end of the proposed *tnsABC* mRNA (Gay *et al.*, 1986). Thus, it seems unlikely to us that *trans* inhibition could reflect the expression of a diffusible transposition inhibitor such as a truncated *tns* polypeptide or, as is the case with transposon Tn*10* (Simons & Kleckner, 1983), an anti-sense RNA.

The products of intact Tn7 transposition are simple insertions rather than cointegrates (Sherratt *et al.*, 1981). We have shown here that miniTn7 elements containing short Tn7 end segments also form simple insertions. Thus, Tn7 does not appear to encode an internal resolution site distinct from its terminal transposition sequences. Several other types of evidence are consistent with the view that Tn7 transposes through the direct formation of simple insertions rather than a two-step reaction involving cointegrate formation and resolution as occurs with transposons of the Tn*3* family (Gill *et al.*, 1978; Heffron *et al.*, 1978). DNA molecules containing two copies of Tn7 are stable and do not resolve (our unpublished observation; Hassan & Brevet, 1983; Hauer & Shapiro, 1984). Also, none of the *tns* genes encodes a protein that acts exclusively to resolve cointegrates (Waddell & Craig, 1988).

How can the organization of Tn7's *cis*-acting transposition sequences be compared to those of other transposable elements? Tn7 is unlike those transposons whose *cis*-acting transposition sequences are provided within terminal inverted repeats (Johnson & Reznikoff, 1983; Sasakawa *et al.*, 1983; Way & Kleckner, 1984; Weinert *et al.*, 1984; Gamas *et al.*, 1985; Huang *et al.*, 1986). The organization of Tn7's transposition sequences is perhaps most reminiscent of bacteriophage Mu, whose ends are also structurally asymmetric and contain multiple recognition sites for MuA transposase (Craigie *et al.*, 1984; Groenen *et al.*, 1985). However, we emphasize that there are no strong sequence similarities between the end sequences of Mu and Tn7 nor do the Tn7 transposition proteins promote the transposition of miniMu elements (Waddell & Craig, 1988).

(a) *Tn7 transposition to target molecules containing the Tn7 ends*

We have demonstrated here that the presence in a target molecule of miniTn*7* elements composed of short end segments containing the *cis*-acting transposition sequences prevents subsequent transposition of another copy of Tn*7* to this molecule. Such interference is most pronounced when the target site is immediately proximal to the Tn*7* element, as in *attTn7*::Tn*7*, a process we call inactivation of adjacent *attTn7*. The ends of Tn*7* also strongly, but to a lesser degree, inhibit transposition to target sites that lie at a distance from the transposon ends, i.e. provide transposition immunity. In the following sections, we discuss these transposition-related phenomena individually, although it should be noted that they could be mechanistically related.

(i) *Inactivation of* attTn7 *by adjacent Tn7 ends*

The sequences required for *attTn7* target activity lie entirely to one side of the specific point of Tn*7* insertion so that Tn*7* insertion into *attTn7* does not physically disrupt these required sequences (Gringauz *et al.*, 1988). Thus, it might appear that *attTn7*::Tn*7* should be a target for subsequent insertion of another copy of Tn*7*. However, tandem insertions of Tn*7* into *attTn7* have not been observed (Lichtenstein & Brenner, 1981; Hauer & Shapiro, 1984) nor have insertions of miniTn*7*s into *attTn7*::Tn*7* been detected (K. Kubo & N. Craig, unpublished results). How is *attTn7*::Tn*7* protected against multiple Tn*7* insertions? We have shown here that the presence in *attTn7* of miniTn*7* elements containing the Tn*7*R sequences necessary for efficient transposition, such as miniTn*7L166* × *R70* or miniTn*7R70* × *R70*, is sufficient to provide a complete inactivation of *attTn7* (reduction of insertion frequency greater than 100,000-fold). A miniTn*7* element with a Tn*7*R segment containing only part of the *cis*-acting transposition sequences (miniTn*7L166* × *R41*) provides considerable, but not complete, inactivation of *attTn7* (more than 100-fold reduction in insertion frequency). These results suggest to us that the loss of target activity of *attTn7*::Tn*7* is related to the presence of the *cis*-acting transposition sequences within the proximal Tn*7*R end. Perhaps the binding of transposition proteins to the Tn*7*R end prevents an interaction between *attTn7* and a protein necessary for its target activity. The inactivation of *attTn7*::Tn*7* does not apparently result from changes in the primary DNA sequence at the specific point of insertion in *attTn7*::Tn*7*. We have found that a segment containing the sequences that result from Tn*7* insertion into *attTn7*, i.e. a junction between the extreme terminus of Tn*7*R and *attTn7*, can exhibit a target activity similar to that of *attTn7* itself (data not shown).

(ii) *Transposition immunity*

Transposition immunity is the phenomenon in which the presence of one copy of a transposable element in a target DNA molecule inhibits subsequent transposition of another copy of this element into any position in the target replicon. This *cis*-acting process has been observed with Tn*3*-like transposons (Robinson *et al.*, 1977) and bacteriophage Mu (Reyes *et al.*, 1987; Adzuma & Mizuuchi, 1988). It has been shown in these systems that the same *cis*-acting sequences are required for transposition and for transposition immunity, and that the presence of a single end of the transposon in the target is sufficient to provide immunity (Adzuma & Mizuuchi; Lee *et al.*, 1983; Arthur *et al.*, 1984; Huang *et al.*, 1986).

There are conflicting reports regarding Tn*7* transposition immunity. Hassan & Brevet (1983) observed that Tn*7* transposed at frequencies similar to those of plasmid RP4 and to its derivative carrying Tn*7* (RP4::Tn*7*). However, Hauer & Shapiro (1984) found that the presence of Tn*7* or miniTn*7L1900* × *R537* (Tn*7*S::Tn*9* Δ *Pst*1 in their nomenclature) in plasmid RP4 substantially reduced the frequency of subsequent Tn*7* insertion in this replicon. As also noted by Hauer & Shapiro, these two studies were carried out under different conditions. For example, Hassan & Brevet analyzed Tn*7* transposition from a plasmid whereas Hauer & Shapiro analyzed transposition from the chromosome. Variations in transposition immunity have been observed under different experimental conditions with other transposable elements (Wallace *et al.*, 1981; Lee *et al.*, 1983; Heritage & Bennett, 1984).

We have observed that Tn*7* displays transposition immunity when it transposes from the chromosome to F-related target plasmids containing miniTn*7* elements. We found that miniTn*7L166* × *R199* provides transposition immunity in both the *tnsABC* + *tnsD* and the *tnsABC* + *tnsE* transposition pathways. Thus, the presence of short Tn*7* end segments containing this element's transposition sequences can make these large target plasmids (greater than 50 kb) immune to further Tn*7* insertion.

Which sequences in miniTn*7L166* × *R199* confer transposition immunity? Our experiments suggest that the *cis*-acting sequences of Tn*7*R that are necessary for efficient transposition are also required for transposition immunity. This view is indicated by our finding that miniTn*7R70* × *R70* confers immunity, whereas miniTn*7R41* × *R41* does not. We also found that miniTn*7* elements that are transposition-defective can provide transposition immunity if they contain an intact Tn*7*R end (as in miniTn*7R41* × *R199*). It remains to be determined if, with Tn*7* as with other transposons, the presence in the target molecule of a single transposon end containing the element's *cis*-acting transposition sequences is sufficient to provide immunity.

If the same sequences in Tn*7*R are involved in transposition and in transposition immunity, why does miniTn*7L166* × *R70*, an element highly active in transposition, fail to provide immunity? Our

interpretation is that R70 lacks sequences that contribute to transposition immunity and to transposition itself. These additional sequences must lie between positions R71 and R141 because miniTn*7L166* × *R141* does effectively provide immunity. We suggested above that the 22 bp repeats in Tn7L and Tn7R are essential transposition signals. R70 contains only three of the Tn7R's four 22 bp repeats whereas R141 contains all four of these repeats. Perhaps the presence of all four 22 bp repeats allows a much more effective association of transposition proteins with Tn7R. Transposition immunity may be a more sensitive reflection of the interaction between the transposon ends and transposition proteins under our experimental conditions. Moreover, immunity effects by Tn7L end segments might even be detectable under other conditions.

What is the mechanism of transposition immunity? Adzuma & Mizuuchi (1988) have shown that transposition immunity in bacteriophage Mu reflects the inability of MuB, a transposition protein required for efficient capture of the target DNA molecule, to bind effectively to a target DNA containing the transposase MuA bound to a transposon end. A somewhat different view suggests that transposition immunity of Tn*3*-like transposons involves an inspection of a target molecule by the incoming transposition complex and subsequent dissociation of the latter when a transposon end segment in the target molecule is encountered (Lee *et al.*, 1983; Sherratt *et al.*, 1983). An attractive hypothesis is that with Tn*7*, as with bacteriophage Mu, transposition immunity reflects the inability of Tn*7*'s target proteins TnsD and TnsE to effectively interact with target DNA molecules containing Tn*7* end segments.

The relationship between transposition immunity, i.e. the ability of a transposon end to inhibit subsequent transposon insertion at a distant target site, and inactivation of adjacent *attTn7*, a target very close to a transposon end, is not known. We have observed that the inhibition of subsequent Tn*7* insertion is much stronger when the target site is immediately proximal to the Tn*7* end as in the inactivation of adjacent *attTn7*. Transposition immunity and adjacent *attTn7* inactivation might both reflect the same process whose potency is distance-dependent and much more pronounced at very short distances. Alternatively, adjacent *attTn7* inactivation may involve a mechanism distinct from that of transposition immunity. For example, the binding of a transposition protein to Tn7R might directly occlude the binding site of a protein required for *attTn7* target activity.

5. Summary

We have shown that the *cis*-acting sequences at the ends of Tn*7* required in transposition are extensive and that Tn7L and Tn7R are structurally as well as functionally different. In addition, we have shown that the Tn7R end sequences that participate directly in Tn*7* transposition also mediate other transposition-related processes such as *trans* inhibition of transposition by multi-copy Tn7R ends and the inactivation of target DNA molecules by transposition immunity and adjacent inactivation. In transposition as well as in these transposition-related phenomena, Tn7R is essential while Tn7L is dispensable.

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