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Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes

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The bacterial transposon Tn7 is capable of high-frequency transposition to a specific site in the *Escherichia coli* chromosome, *attTn7*, and of low-frequency transposition to sites other than *attTn7*. Using an in vitro insertional mutagenesis procedure, we have identified and characterized five *tns* (*Tn* seven) genes that are essential for Tn7 transposition. Three of these genes, *tnsA*, *tnsB*, and *tnsC*, are required, but are not sufficient, for all Tn7 transposition events. In addition, *tnsD* is specifically required for transposition to *attTn7*, whereas *tnsE* is specifically required for transposition to other sites. Thus, Tn7 is an elaborate transposon that encodes two distinct but overlapping transposition pathways.

[Key Words: Tn7; transposition; recombination; in vitro mutagenesis]

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Transposons are DNA segments that have the capacity to move from one position to another within a genome. They encode proteins, generally one or two, that are required for transposition and *cis*-acting DNA sequences, often inverted repeats, that define their termini (for review, see Kleckner 1981; Shapiro 1983; Grindley and Reed 1985). Many bacterial transposons also encode other genetic determinants such as antibiotic resistances. Most transposable elements transpose to many different sites within a genome and, thus, can occasionally insert into genes essential to their host. Perhaps to minimize this deleterious event, transposition for most elements is a low-frequency, tightly regulated event.

The transposon Tn7 (Barth et al. 1976), which encodes resistance to trimethoprim and to spectinomycin and streptomycin, is remarkable in that it transposes at high frequency to a specific chromosomal site in many different bacteria (Hermalsteens et al. 1980; Thomson et al. 1981; Ely 1982). In *Escherichia coli*, where Tn7 transposition has been studied most extensively, this site, called *attTn7*, is located at minute 84 of the chromosome (Barth et al. 1976; Lichtenstein and Brenner 1981, 1982; Walker et al. 1984). The specific point of Tn7 insertion lies within the transcription terminator of *glmS* (Gay et al. 1986; E. Gringauz, K. Orle, C. Waddell, and N. Craig, in prep.), a gene involved in cell-wall biosynthesis. Because Tn7 insertions in *attTn7* do not physically disrupt the *glmS*-coding region, high-frequency transposition to this site can be tolerated by the cell. Chromosomal DNA segments containing *attTn7* also serve as targets for high-frequency, site-specific Tn7 transposition when they are introduced into plasmids (Lichtenstein and Brenner 1981; Rogers et al. 1986;

McKown et al. 1988). The ends of Tn7 show no sequence homology to *attTn7* (Lichtenstein and Brenner 1982; Gay et al. 1986; McKown et al. 1988; E. Gringauz, K. Orle, C. Waddell, and N. Craig, in prep.). Tn7 also transposes at low frequency into many different sites in plasmids (Barth and Grinter 1977; Barth et al. 1978).

In early experiments to identify the transposition genes encoded by Tn7, several groups examined the effect of internal deletions of Tn7 on transposition (Hauer and Shapiro 1984; Smith and Jones 1984; Quarts et al. 1985). These studies clearly established that (1) all required transposition genes are encoded within a 9-kb region of the 14-kb Tn7 transposon (2) at least three separate genes are encoded within this region, and (3) Tn7 transposition proteins, unlike those of many IS elements (e.g., Isberg and Syvanen 1981; Morisato et al. 1983), complement efficiently in *trans*. Hauer and Shapiro (1984) demonstrated that one of the transposition genes is dispensable for transposition from plasmids to the chromosomal *attTn7* site.

We chose to define the transposition genes of Tn7 by insertional mutagenesis, an approach that allows finer dissection of a region than does deletion analysis. Our studies revealed a surprisingly complex array of genes. We have identified five *tns* (*Tn* seven) transposition genes and two distinct but overlapping pathways for Tn7 transposition. *tnsA*, *tnsB*, and *tnsC* are required for all transposition events but are unable to promote transposition in the absence of *tnsD* or *tnsE*. High-frequency transposition to *attTn7* is promoted by *tnsABC* + *tnsD*, whereas low-frequency transposition to sites other than *attTn7* is promoted by *tnsABC* + *tnsE*. Thus, *tnsD* and *tnsE* determine the transposition pathway used by Tn7.

Concurrent with our own studies, Rogers et al. (1986) also identified *tnsA*–*tnsE* using complementation tests between a collection of Tn7 restriction fragments. The *tns* designation was agreed upon by our two groups because it does not imply mechanism or function. It is not clear which *tns* gene, or genes, encodes the enzymatic activity commonly associated with ‘transposases’; i.e., the precise joining of the transposon’s ends to target DNA sequences.

Results

Characterization of the *tns* plasmid pCW4

We have developed a transposition system consisting of three components: (1) a plasmid, pCW4, containing the Tn7 transposition genes, (2) a transposition-defective Tn7 derivative containing the ends of Tn7, and (3) a conjugable plasmid that serves as the target for transposition. pCW4 contains the rightward 9 kb of Tn7 and 165 bp of flanking *attTn7* sequence inserted into the small, multicopy vector pACYC184 (Chang and Cohen 1978). The Tn7 fragment in pCW4 is itself unable to transpose because it does not contain both left and right end sequences of Tn7. pCW4 was assayed for its ability to promote the transposition of a Tn7-end derivative in *trans*. One of two such end derivatives was used: miniTn7Cm (Hauer and Shapiro 1984) or miniTn7Km (L. Arciszewska and N. Craig, in prep.). Both derivatives contain all the essential *cis*-acting sequences at their termini required for transposition and lack any intact transposition gene. Transposition of a Tn7-end derivative from the chromosomal *attTn7* site to a conjugable plasmid was determined by a mating-out assay. One of two conjugable plasmids was used as the target for transposition: (1) pOX38-Gen,*attTn7* (L. Arciszewska and N. Craig, in prep.), hereafter referred to as pOX-*attTn7*, to evaluate transposition to *attTn7*, or (2) pOX38-Gen (Johnson and Reznikoff 1984), hereafter referred to as pOX, to examine transposition to other sites.

As shown in Table 1, transposition of a Tn7-end element to both target plasmids is dependent on pCW4 (lines 2 and 3). In the presence of pCW4, the Tn7-end element transposes at approximately the same frequency as does intact Tn7 (compare lines 1 and 2). Transposition to pOX-*attTn7* occurs at a frequency approximately 50- to 75-fold higher than to pOX (lines 1 and 2), demonstrating the activity of *attTn7*. Thus, the Tn7 transposition genes in pCW4 and a Tn7-end element in *trans* mimic transposition of intact Tn7.

To further characterize pCW4-promoted transposition events, products from transpositions to both pOX-*attTn7* and pOX were analyzed physically to determine the distribution of insertions within the target plasmids. All transpositions to pOX-*attTn7* occurred into the same restriction fragment (Fig. 1, lanes 1–4), consistent with site-specific transposition to *attTn7*. In contrast, transpositions to pOX occurred into several different restriction fragments (Fig. 1, lanes 5–8), indicating low specificity of insertion. These results, together with the data in Table 1, demonstrate that pCW4 contains all the

Table 1. *Tn7* and pCW4-promoted transposition

Tn7 element	Plasmid	Target plasmid ^a	
		pOX	pOX- <i>attTn7</i>
Tn7 ^b	—	1.7×10^{-4}	9.4×10^{-3}
miniTn7Cm ^c	pCW4	1.5×10^{-4}	1.1×10^{-2}
miniTn7Cm ^d	pACYC184	$<2.2 \times 10^{-8}$	$<7.2 \times 10^{-9}$

^a Transposition frequencies of Tn7 element from the chromosomal *attTn7* site were determined by a mating-out assay. Plasmids are as indicated. Each number is the average of three to nine independent trials.

^b Numbers from L. Arciszewska and N. Craig (in prep.).

^c Transposition frequencies of miniTn7Cm were approximately the same when the Tn7 fragment in pCW4 was inserted in the opposite orientation (data not shown).

^d pOX-*attTn7* number is the transposition frequency of miniTn7Km.

Tn7-encoded genes required to promote both high-frequency transposition to *attTn7* and low-frequency transposition to other sites.

Mutagenesis of pCW4

To identify the Tn7-encoded genes involved in transposition, pCW4 was subjected to insertional mutagenesis with a miniMu transposon. The element, miniMuΩ, consists of Ω (Prentki and Krisch 1984), a spectinomycin/streptomycin-resistance determinant flanked by transcription and translation termination signals, bounded by the left and right ends of Mu. MiniMuΩ insertions in pCW4 were generated by an *in vitro* reaction in which pCW4 served as the target for miniMuΩ transposition (see Materials and methods for details) and were recovered by transformation into a strain that lacked a Tn7-end element. This precaution was taken to allow recovery of mutations that might have been lethal to a host containing a Tn7-end element.

We have characterized 96 simple insertions of miniMuΩ in pCW4 generated by this procedure. Figure 2 shows the map position and orientation of each of these 96 miniMuΩ insertions as determined by restriction enzyme analysis. Eighteen insertions map in nonessential regions of pACYC184 or in *attTn7*, whereas 78 insertions map within the Tn7 fragment. It is interesting to note the distribution of miniMuΩ insertions within the Tn7 sequences. Although miniMuΩ insertions map throughout the fragment, two regions—between positions 1.7 and 3.5 kb and between positions 6.45 and 7.8 kb—are notably barren. It is tempting to speculate that the paucity of miniMuΩ insertions within these regions reflects properties of the disrupted *tns* genes. Perhaps the majority of insertions in these regions result in the production of truncated Tns polypeptides, lethal to the host. However, it is also possible that the distribution of miniMuΩ insertions reflects features of Mu transposition (Raibaud et al. 1979; Kamp and Kahmann 1981; M. Mizuuchi and K. Mizuuchi, pers. comm.).

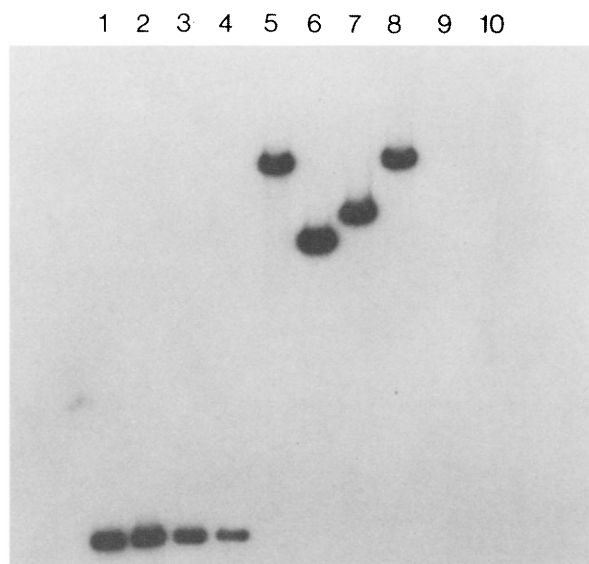


Figure 1. DNA analysis of pCW4-promoted transposition products. Products are from mating-out assays in which miniTn7Km transposed from the chromosomal *attTn7* site to pOX or to pOX-*attTn7*. Each lane is a single transposition product from an independent mating-out experiment. Total DNA was prepared from exconjugants that had acquired Km^R. The DNA was digested with *Eco*RI, size-separated by electrophoresis, and probed with the nick-translated kanamycin fragment of miniTn7Km. *Eco*RI digestion of pOX (59.4 kb in size) yields seven fragments ranging in size from 23.0 to 0.2 kb; there is no *Eco*RI cleavage site within miniTn7Km (1.8 kb in size). Therefore, this analysis is limited to determining the *Eco*RI restriction fragment of pOX into which miniTn7Km transposed. The predicted sizes of miniTn7Km-containing fragments are 24.8, 16.0, 13.1, 10.4, 3.1, 2.6, and 2.0 kb. Bands of approximately 25.5, 17.0, 14.5, and 11.0 kb were observed. Insertions into the three smallest fragments, which represent approximately 4% of the target plasmid, were not observed. pOX-*attTn7* has an additional *Eco*RI site within the *attTn7* cassette; a novel 4.5-kb band was observed in transposition products to pOX-*attTn7*. The *tns* and target plasmids are as indicated. (Lanes 1–4) pCW4/pOX-*attTn7*; (lanes 5–8) pCW4/pOX. No hybridization to pOX-*attTn7* (lane 9) or to pOX (lane 10) was observed in the absence of a miniTn7Km insertion.

Analysis of pCW4 :: miniMuΩ derivatives

Eighty-four of the pCW4 :: miniMuΩ plasmids were introduced into strains containing a Tn7-end element by transformation. These plasmids represent most of the insertions mapping within Tn7 and *attTn7* sequences and three insertions mapping within pACYC184 sequences. With all 84 pCW4 :: miniMuΩ plasmids, transformants were recovered that had no detectable growth defects. Therefore, none of the miniMuΩ insertions produced mutations conditionally lethal for Tn7-bearing strains. The capacity of each plasmid to promote transposition to pOX and to pOX-*attTn7* was determined by a mating-out assay and evaluated qualitatively. A quantitative determination of the transposition frequency was also performed for a subset of the plasmids.

As expected, miniMuΩ insertions in pACYC184 and in *attTn7* sequences had little or no effect on miniTn7Cm transposition (Fig. 2). Plasmids containing

these insertions promoted miniTn7Cm transposition to pOX and to pOX-*attTn7* at an average frequency of 6.5×10^{-5} and 2.8×10^{-2} , respectively. We also observed that miniMuΩ insertions in Tn7 sequences between positions 7.85 and 8.7 kb (Fig. 2) had no effect on the ability of pCW4 to promote transposition (average transposition frequency to pOX, 9.5×10^{-5} ; to pOX-*attTn7*, 4.7×10^{-2}). Thus, this region of Tn7 has no detectable role in transposition.

In sharp contrast, all other pCW4 derivatives with miniMuΩ insertions in Tn7 sequences between positions 0.1 and 7.8 kb (Fig. 2) were altered in their capacity to promote transposition. These plasmids exhibited one of three distinct mutant phenotypes. One class was defective in transposition to both pOX and pOX-*attTn7*, another was altered only in transposition to pOX, and the third was specifically altered in transposition to pOX-*attTn7*.

All miniMuΩ insertions between positions 0.1 and 4.7 kb (Fig. 2) eliminated transposition of miniTn7Cm to both pOX and pOX-*attTn7*. Transposition of miniTn7Cm was undetectable in the presence of these mutant plasmids— $<1.0 \times 10^{-7}$ and $<79.0 \times 10^{-8}$ to pOX and to pOX-*attTn7*, respectively. Thus, this region encodes proteins required for all Tn7 transposition events. Further analysis of this region has revealed that it encodes three *tns* genes: *tnsA*, *tnsB*, and *tnsC* (see below).

Plasmids with miniMuΩ insertions between positions 6.2 and 7.8 kb (Fig. 2) were altered only in transposition to pOX and define the transposition gene *tnsE*. In the presence of these plasmids, miniTn7Cm transposition to pOX was undetectable (average transposition frequency $<1.8 \times 10^{-7}$), but transposition to pOX-*attTn7* occurred at high, wild-type frequencies (average transposition frequency, 4.5×10^{-2}). These results suggest that the *tnsE* gene product is specifically required for transposition to sites other than *attTn7* and that all transposition events promoted by these *tnsE* mutants occur to *attTn7*. We physically examined transposition products to pOX-*attTn7* promoted by a *tnsE* mutant and found that all insertions occurred into the restriction fragment unique to that target (Fig. 3, lanes 13–16, lane 17), consistent with site-specific transposition to *attTn7*. Thus, *tnsE* mutations have no effect on transposition to *attTn7*.

Two lines of evidence suggest that all insertions with the *TnsE* mutant phenotype identify a single gene: (1) A single polypeptide is encoded by this region whose molecular weight is consistent with the coding capacity of the region (Brevet et al. 1985; Smith and Jones 1986; C. Waddell and N. Craig, unpubl.) and (2) DNA sequence analysis has revealed one open reading frame (ORF) within this region (Smith and Jones 1986) with its amino terminus near position 6.2 kb.

Plasmids with miniMuΩ insertions between positions 5.0 and 6.05 kb (Fig. 2) were specifically altered in transposition to pOX-*attTn7* and define the transposition gene *tnsD*. In the presence of these pCW4 derivatives, miniTn7Cm transposition to pOX-*attTn7* was reduced approximately 50-fold, as compared to wild-type trans-

position levels (average transposition frequency, 2.2×10^{-4}), but transposition to pOX was unaffected (average transposition frequency, 1.2×10^{-4}). In vitro transcription and translation of a fragment containing *tnsD* is consistent with this fragment encoding a single polypeptide (C. Waddell and N. Craig, unpubl.) and suggests that the 40-kD protein observed by Brevet et al. (1985) in maxicell experiments was the TnsD protein.

We considered two explanations for transposition to pOX-*attTn7* observed with *tnsD* mutations: (1) *tnsD* mutations eliminate site-specific transposition to *attTn7*, or (2) *tnsD* mutations affect the frequency, but not the site-specificity, of transposition to *attTn7*. To distinguish between these two explanations, we determined the position of Tn7-end derivative insertions within pOX-*attTn7* promoted by a *tnsD* mutant. These insertions were not found within the restriction fragment diagnostic of transposition to *attTn7* but rather in several different fragments common to both pOX-*attTn7* and pOX (Fig. 3, cf. lanes 9-12 to lanes 1-8 and 17). This result provides strong support for the hypothesis that pCW4 derivatives bearing *tnsD* mutations have lost their capacity to promote transposition to *attTn7* but are unaltered in their ability to promote transposition to other sites.

Thus, we propose that *tnsD* and *tnsE* function as target-specific genes; *tnsD* is uniquely required for transposition to *attTn7*, whereas *tnsE* is uniquely required for transposition to sites other than *attTn7*.

Identification of *tnsA*, *tnsB* and *tnsC*

As noted above, all miniMu Ω insertions between positions 0.1 and 4.7 kb (Fig. 2) eliminated the ability of pCW4 to promote transposition to both pOX and pOX-*attTn7*. To define the *tns* genes within this 4.6-kb region, we performed complementation tests between miniMu Ω insertions in pCW4 and various Tn7 fragments.

DNA sequencing of the first 540 bp of the right end of Tn7 (Tn7R) had revealed an ORF beginning approxi-

mately at position 0.1 kb, with its promoter Tn7R proximal (Gay et al. 1986). Assuming this ORF to encode the amino terminus of *tnsA*, we defined the extent of this gene by determining which pCW4 :: miniMu Ω plasmids could complement the transposition-defective Tn7 fragment deleted for this region. As shown in Figure 4A, pCW4 derivatives containing miniMu Ω insertions to the left of the 0.9-kb *Bgl*III site complemented the deletion; i.e., promoted transposition. Thus, the carboxyl terminus of *tnsA* lies between miniMu Ω insertions 93 (position 0.8 kb) and 19 (position 0.95 kb). In other complementation tests, we found that the *Bgl*III fragment extending from positions 0.04 to 0.9 kb (see Fig. 4) does not provide functional TnsA (data not shown).

The *tnsA* analysis above indicates that one end of *tnsB* must lie between the 0.9-kb *Bgl*III site and miniMu Ω insertion 19. Complementation tests between various pCW4 :: miniMu Ω plasmids and a deletion extending into the *tnsB* gene were performed to determine the extent of *tnsB*. The results indicate that the other end of *tnsB* must lie between miniMu Ω insertions 89 (position 2.85 kb) and 49 (position 3.55 kb) (Fig. 4B).

The complementation test in Figure 4B also revealed a third transposition gene in this region, *tnsC*. *tnsC* is defined by those miniMu Ω insertions between positions 3.5 and 4.7 kb that eliminate pCW4-promoted transposition to both pOX and pOX-*attTn7* (Fig. 2) and that complement the *tnsAB* deletion (Fig. 4B). One end of *tnsC* must lie between miniMu Ω insertions 89 and 49 (Fig. 4B). Smith and Jones (1986) sequenced the *Bam*HI fragment in this region (see Fig. 4) and reported an ORF that starts immediately to the left of the 2.9-kb *Bam*HI site and continues leftward through the fragment. Our results suggest that this ORF encodes the amino terminus of *tnsC*. The capacity of Tn7 fragments to provide functional *tnsABC* (see below) is consistent with the carboxyl terminus of *tnsC* lying between miniMu Ω 39 (position 4.65 kb) and the 4.7-kb *Pvu*II site.

Both the TnsB and TnsC polypeptides have been identified in maxicells (Brevet et al. 1985; C. Waddell and N. Craig, unpubl.) and have molecular weights in good

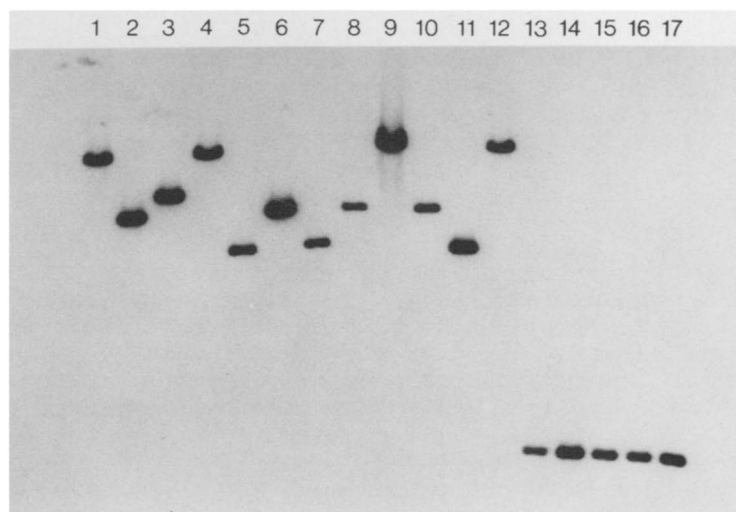


Figure 3. DNA analysis of pCW4 :: miniMu Ω -promoted transposition products. Analysis performed as described in the legend to Fig. 1. The *tns* and target plasmids are as indicated. (Lanes 1-4) pCW4/pOX as in lanes 5-8 of Fig. 1; (lanes 5-8) pCW4 *tnsD* :: miniMu Ω 72/pOX; (lanes 9-12) pCW4 *tnsD* :: miniMu Ω 72/pOX-*attTn7*; (lanes 13-16) pCW4 *tnsE* :: miniMu Ω 78/pOX-*attTn7*; (lane 17) pCW4/pOX-*attTn7* as in lane 1 of Fig. 1.

agreement with the coding capacity of their respective genes. Truncated polypeptides synthesized from various pCW4*tnsB* :: miniMu Ω plasmids suggest that the amino

terminus of *tnsB* is near the *tnsA* gene (C. Waddell and N. Craig, unpubl.).

tns transposition requirements

What are the minimal *tns* requirements for transposition? Are *tnsD* and *tnsE* essential for transposition, or do they act by stimulating *tnsABC*-dependent transposition? Alternatively, does either *tnsD* or *tnsE* have some intrinsic transposition activity in the absence of the other *tns* genes?

To answer these questions, we examined the ability of fragments containing *tnsABC*, *tnsD*, and *tnsE* to promote transposition individually and in combination. We found that none of the individual *tns* fragments can promote transposition of miniTn7Km (Table 2, sections 1–3). The element does transpose, however, when provided with a particular array of *tns* genes and target sequences. *tnsABC* + *tnsD* promote transposition only if the *attTn7* sequence is available (Table 2, section 4); transposition to pOX is not observed. Note that transposition to pOX-*attTn7* promoted by *tnsABC* + *tnsD* (pCW23) approaches 100%. Physical analysis of the transposition products confirmed that all miniTn7Km insertions occurred into the same fragment of pOX-*attTn7* (data not shown), consistent with site-specific transposition to *attTn7*. In contrast, *tnsABC* + *tnsE* promote low-frequency transposition to both pOX and

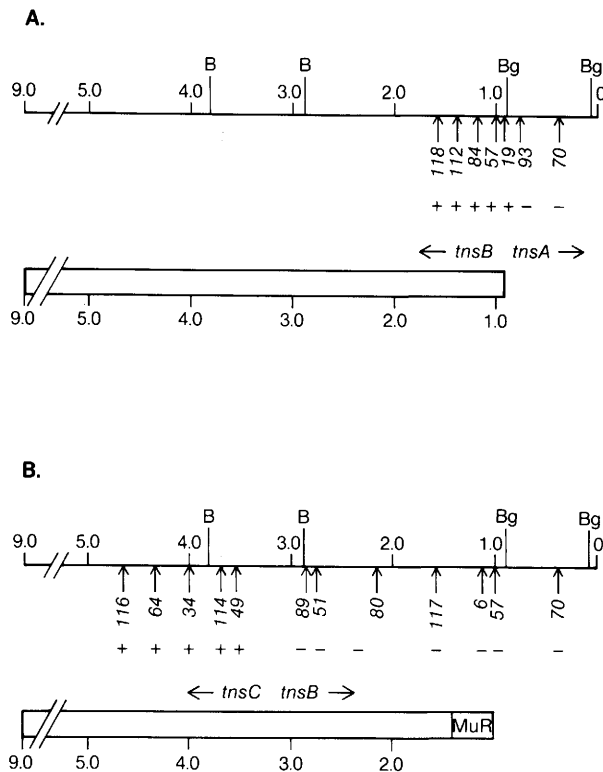


Figure 4. Summary of complementation tests between pCW4 :: miniMu Ω derivatives (lines) and *tns* deletion fragments (open boxes). The lengths of Tn7 sequences are shown in kb; vector sequences are not shown. Only *Bam*HI (B) and *Bgl*III (Bg) restriction sites are shown. The positions of miniMu Ω insertions in pCW4 are indicated by vertical arrows; italicized numbers identify individual insertions. Transposition of miniTn7Cm from the chromosomal *attTn7* site to pOX was measured by a mating-out assay. The ability of each pCW4 :: miniMu Ω insertion to complement the deletion and promote transposition is indicated by a + or a -. Each insertion was assayed in one to three independent trials. (A) Complementation tests against the *tns* deletion in pCW12. pCW12 is deleted for all Tn7 sequences to the right of the 0.9 *Bgl*III site. pCW12 does not promote miniTn7Cm transposition (average transposition frequency $<7.7 \times 10^{-8}$). In these complementation tests, the average - transposition result = $<8.8 \times 10^{-8}$ and the average + transposition result = 9.5×10^{-6} . This transposition frequency is approximately 16-fold lower than pCW4-promoted transposition (Table 1) and probably reflects the instability of pCW12. There is measurable loss of pCW12 even under selective growth conditions; only results of matings in which $\geq 50\%$ of the donors retained pCW12 are shown. (B) Complementation tests against the *tns* deletion in pCW34. pCW34 is deleted for all Tn7 sequences to the right of miniMu Ω insertion 112 (located at position 1.4 kb) and contains the right end of Mu (MuR). pCW34 does not promote miniTn7Cm transposition (average transposition frequency = 3.5×10^{-8}). In these complementation tests, the average - transposition result = 8.0×10^{-8} , and the average + transposition result = 7.5×10^{-5} .

Table 2. *tns* fragment-promoted transposition

<i>tns</i> fragment ^a	Target plasmid ^b	
	pOX	pOX- <i>attTn7</i>
1. <i>tnsABC</i> (pCW15) (pCW16) (pCW21)	$<2.4 \times 10^{-8}$ $<2.9 \times 10^{-8}$ 4.9×10^{-9}	$<4.5 \times 10^{-8}$ $<3.6 \times 10^{-8}$ 5.3×10^{-8}
2. <i>tnsD</i> (pCW23) (pCW25) (pCW26)	$<4.2 \times 10^{-9}$ $<1.9 \times 10^{-8}$ $<4.3 \times 10^{-8}$	$<3.7 \times 10^{-8}$ $<2.5 \times 10^{-8}$ $<6.6 \times 10^{-8}$
3. <i>tnsE</i> (pCW30) (pCW31) (pCW32)	$<4.6 \times 10^{-8}$ $<3.0 \times 10^{-8}$ $<1.2 \times 10^{-8}$	$<9.5 \times 10^{-9}$ $<4.0 \times 10^{-8}$ $<6.8 \times 10^{-8}$
4. <i>tnsABC</i> + <i>tnsD</i> (pCW15/pCW23) (pCW16/pCW23) (pCW15/pCW25) (pCW16/pCW25)	$<9.9 \times 10^{-9}$ $<7.6 \times 10^{-9}$ $<2.5 \times 10^{-9}$ $<2.8 \times 10^{-9}$	9.1×10^{-1} 1.0 1.5×10^{-1} 9.1×10^{-2}
5. <i>tnsABC</i> + <i>tnsE</i> (pCW15/pCW30) (pCW16/pCW30) (pCW21/pCW30)	4.8×10^{-5} 2.0×10^{-4} 2.2×10^{-4}	2.1×10^{-4} 1.2×10^{-4} n.d.

^a *tns* fragments were tested in different vectors and in different orientations with respect to external vector promoters. pCW23, pCW25, and pCW30 are derivatives of pUC18; all other *tns* plasmids are derivatives of pACYC184.

^b Transposition frequencies of miniTn7Km from the chromosomal *attTn7* site were determined by a mating-out assay. *tns* and target plasmids are as indicated. Except for *tnsABC*, each number is the average of three to five independent trials. The *tnsABC* numbers are the result of a single trial. (n.d.) Not determined.

pOX-*attTn7* (Table 2, section 5). These miniTn7Km insertions were found in the fragments common to both target plasmids but never in the fragment unique to pOX-*attTn7* (data not shown). We conclude that Tn7 transposition requires *tnsD* or *tnsE* in addition to *tnsA*, *tnsB*, and *tnsC* and that *tnsD* and *tnsE* specifically promote Tn7 transposition to different target sequences.

Genetic organization of the *tns* genes

Are the *tns* genes organized in an operon or as separate transcription units? The analysis presented below is consistent with there being four transcription units for the five *tns* genes: *tnsA* and *tnsB* comprise an operon, and *tnsC*, *tnsD*, and *tnsE* are transcribed independently. The transcription units were identified based on the ability of miniMu Ω insertions to exert polar effects on downstream genes in an operon; Ω is a strongly polar element (Prentki and Krisch 1984; Frey and Frisch 1985).

A fragment containing *tnsA* was unable to complement a *tnsA*::miniMu Ω mutation in *trans* (Table 3, line 3) but did complement a *tnsA* deletion (line 2). This result suggests that the *tnsA*::miniMu Ω mutation is polar on *tnsB* and, therefore, that *tnsA* is the first gene in an operon. Presumably, the *tnsA* deletion is not polar on *tnsB* because *tnsB* is transcribed from an external promoter provided by the vector. Sequence analysis of *tnsA* (Gay et al. 1986; K. Orle and N. Craig, unpubl.) and part of *tnsB* (K. Orle and N. Craig, unpubl.) reveals that both genes are transcribed in the same direction, consistent with a *tnsAB* operon. The transcriptional organization of *tnsA* and *tnsB* does not negate our conclusion that *tnsA* is essential for transposition: a wild-type copy of *tnsA* is required in *trans* to compensate for *tnsA* deletions (Table 3, line 2; Fig. 4A).

Does the *tnsAB* operon include *tnsC*? The complementation results in Table 3, column 3, suggest that it does not. A fragment containing *tnsAB* could comple-

Table 3. Effects of polar insertions in *tnsA* and *tnsB*

pCW4 derivative	Complementing plasmid ^a		
	None	<i>tnsA</i> (pCW43)	<i>tnsAB</i> (pCW45)
pCW4	4.4×10^{-4}	2.5×10^{-5}	2.8×10^{-5}
Δ <i>tnsA</i> (pCW51)	$<1.3 \times 10^{-8}$	2.4×10^{-6}	5.7×10^{-6}
<i>tnsA</i> ::miniMu Ω 81	$<7.7 \times 10^{-8}$	2.4×10^{-8}	1.6×10^{-5}
<i>tnsB</i> ::miniMu Ω 57	$<6.9 \times 10^{-8}$	n.d.	2.9×10^{-5}
<i>tnsB</i> ::miniMu Ω 11	$<3.3 \times 10^{-8}$	n.d.	7.1×10^{-6}
<i>tnsB</i> ::miniMu Ω 89	n.d.	n.d.	6.0×10^{-6}
<i>tnsC</i> ::miniMu Ω 34	$<1.4 \times 10^{-7}$	n.d.	$<7.4 \times 10^{-9}$

^a Transposition frequencies of miniTn7Km from the chromosomal *attTn7* site to pOX were determined by a mating-out assay. *tns* plasmids are as indicated. pCW4::miniMu Ω numbers from Fig. 2. All other numbers are the average of three to five independent trials. n.d. = not determined. We note that *tnsA*- and *tnsAB*-complemented transposition frequencies are ≥ 15 -fold lower than pCW4-promoted transposition. This may be an effect of multicopy Tn7 right end sequences (L. Arciszewska and N. Craig, in prep.) or multicopy *TnsA*.

Table 4. Effects of polar insertions in *tnsC*

<i>tnsCD</i> plasmid ^a	<i>tnsABC</i> ^b (pCW15)
<i>tnsCD</i> (pCW52)	1.5×10^{-2}
<i>tnsC</i> ::miniMu 34 <i>tnsD</i> (pCW54)	2.4×10^{-3}
<i>tnsC</i> ::miniMu 39 <i>tnsD</i> (pCW55)	1.5×10^{-4}
<i>tnsCD</i> (pCW53)	3.9×10^{-1}
<i>tnsC</i> ::miniMu 39 <i>tnsD</i> (pCW56)	1.1×10^{-2}
—	$<4.5 \times 10^{-8}$

^a In pCW52, pCW54, and pCW55, the *tns* fragment is oriented so that *tnsC* is adjacent to vector *Plac*; in pCW53 and pCW56, *tnsD* is adjacent to vector *Plac*.

^b Transposition frequencies of miniTn7Km from the chromosomal *attTn7* site to pOX-*attTn7* were determined by a mating-out assay. *tns* plasmids are as indicated. To ensure that the transposition frequencies reflected only *tnsD*-promoted events to *attTn7*, *tnsE* was not present in these experiments. *tnsABC* number is from Table 2. All other numbers are the average of three to five independent trials.

ment both a *tnsA* deletion (line 2) and a *tnsA*::miniMu Ω mutation (line 3), as well as three different *tnsB*::miniMu Ω mutations (lines 4–6) and was unable to complement a *tnsC*::miniMu Ω mutation (line 7). These results suggest that the operon is limited to the *TnsA* and *tnsB* genes.

What is the relationship of the *tnsC* and *tnsD* genes? An operon with *tnsD* promoter proximal to *tnsC* is unlikely. *tnsD*::miniMu Ω mutants are unaltered in *tnsE*-dependent transposition (Fig. 2), an event that requires *tnsC*. Moreover, limited sequence analysis of *tnsC* (Smith and Jones 1986) and *tnsD* (K. Orle and N. Craig, unpubl.) suggests that these two genes are transcribed in the same direction but in the opposite orientation predicted for a *tnsDC* operon.

Do the *tnsC* and *tnsD* genes comprise an operon with *tnsC* promoter proximal? We interpret the results in Table 4 to indicate that *tnsC*::miniMu Ω mutants are not polar on *tnsD* and, therefore, that *tnsC* and *tnsD* are separate transcription units. Each of the *tnsC*::miniMu Ω *tnsD* plasmids tested could complement the *tnsABC* plasmid and promote transposition to pOX-*attTn7* (cf. lines 2, 3, and 5 to line 6). However, miniMu Ω insertions in *tnsC* decrease the frequency of transposition (cf. lines 2 and 3 to line 1, and line 5 to line 4). This inhibitory effect was most pronounced for plasmids in which the amino terminus of *tnsC* was proximal to an external vector promoter (lines 1–3) and was markedly less severe for plasmids in which the carboxyl terminus of *tnsD* was promoter proximal (lines 4 and 5). Because the observed orientation effect is inconsistent with a *tnsCD* operon, we suggest that the decreased transposition frequencies reflect properties of the plasmid constructions. Perhaps an in-frame *TnsC* fusion protein or an abundance of a truncated *TnsC* polypeptide that has an inhibitory effect in *trans* was created in these plasmids.

Direct examination of *TnsD* and *TnsE* mutant phenotypes indicate that these two genes are transcribed independently of one another. miniMu Ω insertions in either

gene have no effect on the transposition pathway promoted by the other (see Fig. 2).

Effect of increased copy number of *tns* genes on Tn7 transposition

Transposition of intact Tn7 was measured in the presence of plasmids containing a *tnsD* or a *tnsE* fragment. Tn7 transposition to pOX decreased approximately five-fold in the presence of *tnsD* plasmids (Table 5), whereas transposition to pOX-*attTn7* decreased approximately five-fold in the presence of two *tnsE* plasmids (Table 5, pCW31 and pCW32). It is tempting to speculate that we have created cellular conditions in which TnsD and TnsE are competing for some limiting component of the transposition machinery. pCW4 had no significant effect on Tn7 transposition (data not shown).

The results in Table 5 also show that none of the *tnsD* or *tnsE* plasmids significantly increased Tn7 transposition to either target plasmid. Smith and Jones (1984) found that the *tnsE* gene in multicopy stimulated Tn7 transposition approximately 30-fold. We note that several features of the two studies differ; perhaps different rate-limiting determinants were measured. Although our assays measured transposition of Tn7 from chromosomal *attTn7* to an IncFI conjugable plasmid (pOX), whereas Smith and Jones measured transposition of Tn7 from a site in the multicopy plasmid ColE1 to an IncP conjugable plasmid. The ColE1 insertion site shows no sequence homology to *attTn7* (Lichtenstein and Brenner 1982), suggesting that the original transposition event was promoted by *tnsE* (K. Kubo and N. Craig, in prep.). Experiments measuring transposition from a *tnsE* site in an F' episome demonstrated that transposition from a *tnsE* site requires the same set of *tns* genes as does transposition from *attTn7* (N. Craig and C. Waddell, unpubl.). However, these experiments do not address the level of Tns proteins required in the two different reactions.

Discussion

Our analysis of the transposition genes of Tn7 has added to this transposon's list of distinguishing characteristics. We have identified a surprisingly complex array of five

Table 5. Tn7 transposition in the presence of multicopy *tnsD* and *tnsE*

<i>tns</i> plasmid	Target plasmid ^a	
	pOX	pOX- <i>attTn7</i>
— ^b	1.7×10^{-4}	9.4×10^{-3}
<i>tnsD</i> (pCW23)	2.6×10^{-5}	2.2×10^{-2}
(pCW25)	4.2×10^{-5}	1.2×10^{-2}
(pCW26)	2.5×10^{-5}	1.6×10^{-2}
<i>tnsE</i> (pCW30)	5.5×10^{-5}	5.3×10^{-3}
(pCW31)	5.3×10^{-5}	1.6×10^{-3}
(pCW32)	4.7×10^{-5}	1.8×10^{-3}

^a Transposition frequencies of Tn7 from the chromosomal *attTn7* site were determined by a mating-out assay. *tns* and target plasmids are as indicated. Each number is the average of three to nine independent trials.

^b Numbers from L. Arciszewska and N. Craig (in prep.)

tns genes, *tnsA*, *tnsB*, *tnsC*, *tnsD*, and *tnsE*, that have essential roles in transposition. No other characterized transposon has such an elaborate array of transposition genes; most other transposons encode one or two proteins with essential transposition functions (reviewed in Kleckner 1981; Shapiro 1983; Grindley and Reed 1985). Our study of the *tns* genes employed a novel insertional mutagenesis procedure. In an in vitro Mu transposition reaction, a polar miniMu Ω element was transposed to sequences within the *tns* plasmid pCW4. Analysis of the disrupted pCW4 derivatives led to the identification and characterization of the *tns* genes. Among the many useful features of this mutagenesis procedure are the large number of independent insertions produced in a single in vitro reaction, the creation of many different antibiotic-resistant insertions throughout the target DNA sequences, and the potential use of these insertions as convenient priming sites for DNA sequence analysis (Adachi et al. 1987). The position and orientation of the five *tns* genes within Tn7 are diagrammed in Figure 5. All five *tns* genes are transcribed in the same direction. Our analysis of polar effects of the *tns* :: mini-Mu Ω insertions is consistent with the hypothesis that

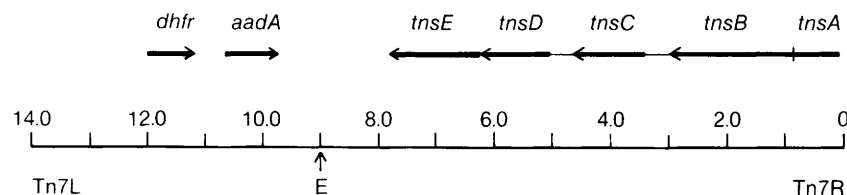


Figure 5. Diagram of Tn7. Tn7L and Tn7R designate the left and right ends of Tn7, respectively. Numbers indicate the length of Tn7 in kb. The position of the unique *EcoRI* site (E) in Tn7 is indicated by the vertical arrow. Horizontal arrows indicate the position and direction of transcription of the Tn7-encoded genes. *dhfr* encodes a dihydrofolate reductase (Fling and Richards 1983) that confers resistance to trimethoprim, and *aadA* encodes a 3'-(9)-*O*-nucleotidyltransferase (Fling et al. 1985) that provides resistance to streptomycin/spectinomycin. *tnsA*-*tnsE* are transposition genes of Tn7. The boundaries and directions of these genes were determined by the analysis of miniMu Ω insertions in pCW4 and of fragments containing *tns* genes (thick lines) and limited sequence analysis (thin lines) (Smith and Jones 1986; K. Orle and N. Craig, unpubl.).

tnsA and *tnsB* comprise an operon, whereas *tnsC*, *tnsD*, and *tnsE* are separate transcription units. We note, however, that substantial polar effects on *tns* gene expression may not have been detected in our transposition assays. A definitive view of the transcriptional organization of the *tns* genes must await direct analysis of the *tns* transcripts.

The role of *tnsD* and *tnsE* in transposition

The large number of *tns* genes is explained in part by the finding that Tn7 can participate in either of two transposition pathways in which transposition is directed by one of two target-specific genes, *tnsD* or *tnsE*. Tn7-end derivatives transpose efficiently and exclusively to *attTn7* when provided *tnsA*, *tnsB*, *tnsC*, and *tnsD*. If *tnsE* is substituted for *tnsD*, other target sites are used at a low frequency and *attTn7* is ignored. *tnsA*, *tnsB*, and *tnsC* are absolutely required in both transposition pathways. A similar view of the roles of *tnsD* and *tnsE* in target selection has emerged in studies in which the *E. coli* chromosome serves as the transposition target (K. Kubo and N. Craig, unpubl.). Rogers et al. (1986) also showed that *tnsD* and *tnsE* promote transposition to different target sites; however, they did not demonstrate a requirement for *tnsA*, *tnsB*, and *tnsC* in *tnsE*-promoted transposition. It will be interesting to determine how the TnsD and TnsE proteins select Tn7 target sites and what role these proteins have in establishing the frequency of transposition in the two transposition pathways.

The existence of target-specific proteins is another novel feature of Tn7 transposition. In the transposition of phage Mu, one of two element-encoded transposition proteins, Mu B, binds nonspecifically to DNA (Chaconas et al. 1985) and presumably interacts with target DNA. Although the Mu B protein may facilitate Mu end capture by target DNA molecules, its participation in the transposition reaction differs from that of TnsD and TnsE in two important respects: Mu B does not direct selection of a specific target sequence and some Mu transposition can occur in the absence of Mu B (R. Craigie, M. Mizuuchi, and K. Mizuuchi, pers. comm.).

The role of *tnsB* in transposition

tnsB mutants are completely defective in both Tn7 transposition pathways and, therefore, must lack a function common to all transposition events. We believe this function is utilization of *cis*-acting sequences at the ends of Tn7. An integral step in all transposition models is the identification and cleavage of sequences at the termini of the transposon (Berg 1977; Grindley and Sherratt 1978; Shapiro 1979). Biochemical studies in our laboratory have identified a *tnsB*-dependent DNA-binding activity that specifically recognizes sequences present in both ends of Tn7 (McKown et al. 1987). This activity does not require the gene products of *tnsA*, *tnsC*, *tnsD*, or *tnsE* (McKown et al. 1987). It is not yet known whether TnsB has any other enzymatic activity in addition

to its proposed DNA-binding activity. Binding to specific end sequences has also been observed with MuA of Mu, InsA of IS1, and with transposases of the Tn3 family (Craigie et al. 1984; Wishart et al. 1985; Zerbib et al. 1987; L. Wiater and N. Grindley, pers. comm.). Several lines of evidence suggest that TnsB also has a regulatory role in *tns* gene expression (Rogers et al. 1986; McKown et al. 1987).

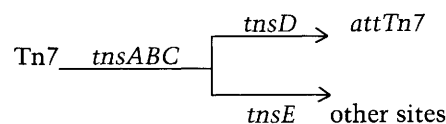
The role of *tnsA* and *tnsC* in transposition

Our studies have demonstrated that both *tnsA* and *tnsC* are essential for all Tn7 transposition events but have not defined specific functions for the *tnsA* and *tnsC* gene products. We can exclude the possibility that one of these gene products acts only as a site-specific recombinase to resolve cointegrate transposition products. Neither a *tnsA* deletion nor disruptions in any of the *tns* genes resulted in cointegrate transposition products when we assayed miniTn7Cm transposition from a plasmid (C. Waddell and N. Craig, unpubl.).

How might the *tnsA* and *tnsC* gene products participate in transposition? Although many models can be evoked, three particularly intriguing possibilities are worthy of mention. Perhaps one of these proteins functions in synapsis of donor and target sequences by serving as a 'linker protein' that recognizes both TnsB and the target proteins TnsD and TnsE. This model is analogous to the role of P protein in λ replication (reviewed in Furth and Wickner 1983). Perhaps, the transposase enzymatic activity of Tn7 is not encoded in a single gene but is produced upon the formation of a multicomponent Tns protein complex. Several transposition reactions require host proteins such as IHF or HU, in addition to element-encoded proteins (Craigie et al. 1985; Morisato and Kleckner 1987). IHF has no detectable role in Tn7 transposition (K. Kubo and N. Craig, unpubl.); perhaps *tnsA* or *tnsC* encodes a functional analog of IHF or of another host protein. These last two scenarios would help account for the surprisingly large number of Tn7 transposition genes.

Concluding remarks

Our identification and characterization of the *tns* genes of Tn7 has led to the following model of Tn7 transposition:



It will be interesting to elucidate the mechanism(s) by which the *tns* gene products mediate Tn7 transposition.

Materials and methods

Media, chemicals, and enzymes

LB broth and agar were used, as described by Miller (1972), except that 1 mg/ml glucosamine was added to agar. Trimetho-

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prim selection was on Isosensitest agar (Oxoid). Antibiotic concentrations used were 100 μ g/ml carbenicillin (Cb), 30 μ g/ml chloramphenicol (Cm), 10 μ g/ml gentamycin (Gen), 50 μ g/ml kanamycin (Km), 20 μ g/ml nalidixic acid (Nal), 50 or 500 μ g/ml spectinomycin (Sp), 50 μ g/ml streptomycin (St), 5 or 20 μ g/ml tetracycline (Tc), and 100 μ g/ml trimethoprim (Tp). DNA-modifying enzymes were obtained from commercial sources and used as recommended by the manufacturer.

Strains

NLC51 is F⁻ *araD139* Δ (*argF-lac*)U169 *rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR val^R recA56* (McKown et al. 1987). CW51 is F⁻ *ara⁻ arg⁻ lac proXIII nal^R rif^R recA56* and was obtained by replacing F'*lacI^QL8 pro⁺* in NK5830 (N. Kleckner, Harvard University) with F'*ts lac⁺* :: Tn7 by plasmid exclusion and isolating a Tn7^{lac⁻} derivative after growth at 42°C. All other strains in this work are derivatives of NLC51, in which (1) Tn7, or a Tn7-end element, occupies the chromosomal *attTn7* site, (2) a conjugable plasmid, pOX38-Gen or pOX38-Gen, *attTn7*, is present, and (3) one or two *tns* plasmids is present. Chromosomal *attTn7* insertions were obtained as follows. Tn7, or a Tn7-end element, was introduced on a conjugable plasmid into NLC51 or NLC51 pCW4, respectively. The element-bearing plasmid was lost either by plasmid exclusion or by growth under nonpermissive conditions, and isolates that retained the Tn7-end element-encoded drug resistance were recovered. Insertions into the chromosomal *attTn7* site were verified by P1 transduction or by physical analysis using Southern blots (data not shown). Plasmids were introduced into strains following standard conjugation (Miller 1972) or transformation (Maniatis et al. 1982) techniques.

Tn7 derivatives

The derivative of Tn7 used in this laboratory, Tn7S, contains an IS1 element near the drug-resistance determinants of Tn7; however, the transposition properties of Tn7S are indistinguishable from those of canonical Tn7 (Hauer and Shapiro 1984). miniTn7Cm is Tn7S :: Tn9 Δ PstI (Hauer and Shapiro 1984) and contains approximately 1.9 kb of the left end of Tn7 and 537 bp of the right end of Tn7, flanking the chloramphenicol-resistance determinant of Tn9. miniTn7Km (McKown et al. 1988) contains 166 bp of the left end of Tn7 and 199 bp of the right end of Tn7, flanking a segment encoding kanamycin resistance. miniTn7Km contains all the essential sequences at the termini of Tn7 required for transposition (L. Arciszewska and N. Craig, in prep.).

Manipulation and characterization of DNA

Plasmid growth, isolation, restriction enzyme analysis, and transformation were performed as described by Maniatis et al. (1982). Recombinant molecules were made using standard cloning techniques, described by Maniatis et al. (1982), except that usually DNA fragments were contained in slices from low-melting-temperature agarose gels (Sea Plaque), as described by Struhl (1983).

Plasmids

pOX38-Gen (Johnson and Reznikoff 1984) is a transfer proficient derivative of the conjugable plasmid F and lacks $\gamma\delta$ or IS elements. pOX38-Gen, *attTn7* (L. Arciszewska and N. Craig, in prep.) is the same as pOX38-Gen, except that it carries a transposition-defective, tetracycline-resistant derivative of Tn10 that contains *attTn7*.

See Figures 2 and 6 for the location of restriction sites and miniMu Ω insertions utilized in formation of the following plasmids.

pCW4 was described previously (McKown et al. 1987) and contains approximately 9 kb of Tn7, extending from the unique *EcoRI* site at position 9.0 through the right end of Tn7 and 165 bp of flanking *attTn7* sequence inserted into the *EcoRI* site of pACYC184, with *attTn7* near vector *Pcat*.

pCW10. The *EcoRI*-*BglIII* fragment containing *tnsA*-*tnsE* was inserted into *EcoRI*-*BamHI*-digested pUC19.

pCW12. The *EcoRI*-*BglIII* fragment containing *tnsB*-*tnsE* was inserted into *EcoRI*-*BamHI*-digested pUC19.

pCW15 and pCW16. The *PvuII*-*PvuII* fragment from pCW4 containing *tnsA*, *tnsB*, and *tnsC* was inserted into the Klenow-treated *SalI* site of pACYC184, with *tnsC* near vector *Ptc* (pCW15) or with *tnsA* near vector *Ptc* (pCW16).

pCW21. The *EcoRI*-*EcoRI* fragment from pCW4 :: miniMu Ω 47 containing *tnsA*, *tnsB*, and *tnsC* was inserted into the *EcoRI* site of pACYC184, with *tnsA* near vector *Pcat*.

pCW23, pCW25, and pCW26. The *HpaI*-*EcoRI* fragment from pCW4 :: miniMu Ω 39 containing *tnsD* was treated with Klenow fragment to repair the *EcoRI* end and was inserted into the *HincII* site of pUC18 with the Mu sequences adjacent to vector *Plac* (pCW23) or with the *HindIII* site near vector *Plac* (pCW25), or into the Klenow-treated *SalI* site of pACYC184, with the *HindIII* site near vector *Ptc* (pCW26).

pCW30, pCW31, and pCW32. The *HindIII*-*HindIII* fragment from pCW4 containing *tnsE* was inserted into the *HindIII* site of pUC18, with the *BstEII* site near vector *Plac*, (pCW30) or into

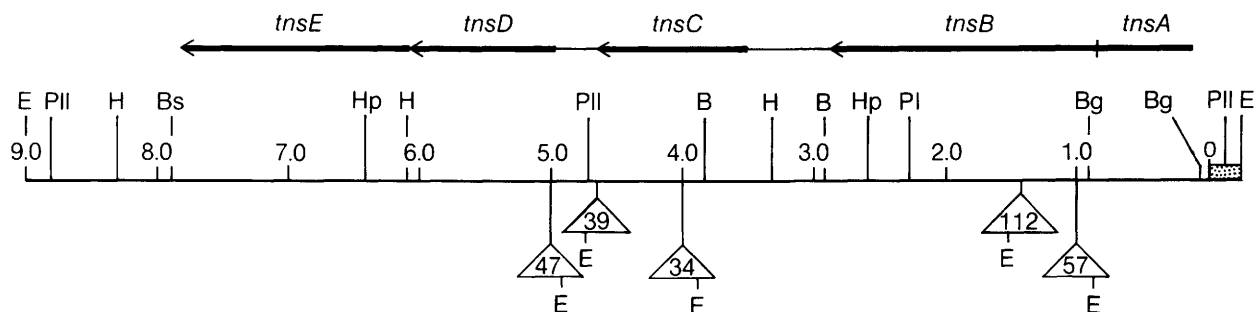


Figure 6. Diagram of Tn7 fragment in pCW4. The line represents Tn7 sequences, and the speckled box designates flanking *attTn7* DNA. The *tns* genes are shown as in Fig. 5. The positions of miniMu Ω insertions in pCW4 derivatives used in the formation of recombinant plasmids are indicated. The miniMu Ω are not drawn to scale, and only the *EcoRI* site is shown. A partial restriction map of the Tn7 fragment is also shown. (B) *BamHI*; (Bg) *BglIII*; (Bs) *BstEII*; (E) *EcoRI*; (H) *HindIII*; (Hp) *HpaI*; (PI) *PvuI*; (PII) *PvuII*.

the *Hind*III site of pACYC184, with the *Bst*EIII site near vector *Ptc* (pCW31) or with the *Hpa*I site near vector *Ptc* (pCW32).

pCW34. The *Eco*RI–*Eco*RI fragment from pCW4 :: miniMuΩ 112 containing *tnsE*, *tnsD*, *tnsC*, and part of *tnsB* was inserted into the *Eco*RI site of pUC18, with the Mu sequences adjacent to vector *Plac*.

pCW43. The *Eco*RI–*Bgl*II fragment from pCW4 :: miniMuΩ 57 containing the *tnsA*, *tnsB* junction and the *Bgl*II–*Hind*III fragment from pCW10 containing most of *tnsA* were inserted into *Eco*RI–*Hind*III-digested pUC19.

pCW45. The *Hind*III–*Hind*III fragment from pCW10 containing *tnsA* and *tnsB* was inserted into the *Hind*III site of pUC18, with *tnsA* near vector *Plac*.

pCW51. The *Bgl*II–*Bgl*II fragment of pCW4 containing most of *tnsA* was deleted.

pCW52 and pCW53. The *Hpa*I–*Hpa*I fragment from pCW4 containing *tnsC* and *tnsD* was inserted into the *Hinc*II site of pUC18, with *tnsC* near vector *Plac* (pCW52) or with *tnsD* near vector *Plac* (pCW53).

pCW54. The *Hpa*I–*Hpa*I fragment from pCW4 :: miniMuΩ 34 containing *tnsC* :: miniMuΩ 34 and *tnsD* was inserted into the *Hinc*II site of pUC18, with *tnsC* :: miniMuΩ 34 near vector *Plac*.

pCW55 and pCW56. The *Hpa*I–*Hpa*I fragment from pCW4 :: miniMuΩ 39 containing *tnsC* :: miniMuΩ 39 and *tnsD* was inserted into the *Hinc*II site of pUC18, with *tnsC* :: miniMuΩ 39 near vector *Plac* (pCW55) or with *tnsD* near vector *Plac* (pCW56).

miniMuΩ transposition reaction

The miniMuΩ plasmid pMK386 was made by Dr. Robert Craigie (NIH). pMK386 is the same as pMK26 (a derivative of pMK108) (Mizuuchi 1983; Craigie and Mizuuchi 1986), except that the *Bam*HIΩ fragment from pHP45Ω (Prentki and Krisch 1984) is inserted at the *Bam*HI site in the Mu part of pMK26.

The miniMuΩ transposition reaction was carried out by R. Craigie. pMK386 and pCW4 were the DNA substrates for the in vitro Mu DNA strand-transfer reaction, as described in Craigie et al. (1985). The resulting transposition intermediates were resolved, as described in Craigie and Mizuuchi (1985). The resolved transposition products were digested with *Aat*II, a restriction enzyme that cleaves pMK386 outside of the miniMuΩ sequences, to linearize cointegrate products, intramolecular rearrangements of pMK386, and unreacted pMK386. There is no *Aat*II site in pCW4. The DNA was then treated with pronase, phenol extracted, ethanol precipitated, and suspended in 30 μl of 10 mM Tris (pH 8.0), 0.5 mM EDTA.

One microliter of a 1:10 dilution was used to transform competent HB101 cells (Boyer and Roulland-Dussoix 1969). Of the 127 Tc^RSp^R transformants tested, 9 were also Cb^R, indicating that pMK386 was present in addition to pCW4. Restriction analysis of plasmid DNA from the 118 Cb^R transformants revealed that 96 of the plasmids contained a single simple insertion of miniMuΩ into pCW4, whereas 22 were the result of multiple miniMuΩ transposition events and were not characterized further. The positions of miniMuΩ insertions were determined by *Pvu*II and *Pvu*I restriction analysis of plasmid DNA; orientation was determined by *Eco*RI or *Sph*I restriction analysis. The map position of each insertion was determined to an accuracy of at least ±200 bp. The positions of various miniMuΩ insertions with respect to Tn7 restriction sites were also determined. Among the sites analyzed were *Bam*HI, *Bgl*II, *Bst*EIII, *Hind*II, and *Hpa*I. The positions of these sites within the Tn7 fragment in pCW4 are diagrammed in Figures 2 and 6. There is no *Sph*I site in the Tn7 fragment, and there are no *Bgl*II, *Hpa*I, or *Pvu*II sites in miniMuΩ.

Transposition assay

Transposition frequencies were determined by a mating-out assay. For each strain tested, individual single colonies were inoculated into LB broth with the appropriate selective antibiotic. Cultures were grown at 37°C to an OD⁶⁰⁰ of approximately 0.4 and used directly or were grown overnight to saturation, subcultured, and grown to the appropriate density. Each culture was mixed with the recipient strain, also grown to an OD⁶⁰⁰ of 0.4, at a ratio of 1 : 10, donor : recipient. The recipient strain was always CW51, and donor strains were derivatives of NLC51 pOX38-Gen or NLC51 pOX38-Gen, *att*Tn7. The mating mixture was incubated at 37°C with gentle aeration. After 60 min, the mixture was vortexed vigorously, placed on ice, and aliquots plated. The total number of exconjugants was determined by selection of Gen^RRif^R colonies. The number of exconjugants that had acquired a Tn7 derivative was determined by selection of Tp^RRif^R, Cm^RRif^R, or Km^RRif^R colonies for Tn7, miniTn7Cm, or miniTn7Km transposition, respectively. The transposition frequency is expressed as the total number of Tn7 derivative-containing exconjugants divided by the total number of exconjugants. In qualitative mating-out assays, the total number of exconjugants was not determined.

Southern blot hybridization analysis

Total DNA was digested with *Eco*RI, electrophoresed in 0.6% agarose, and transferred to Nytran (Schleicher and Schuell) in 10× SSC. Filters were hybridized at 42°C in 50% formamide, 10× Denhart's solution, 0.1% SDS, 0.01% salmon sperm DNA, 0.05% sodium pyrophosphate, 1 mM EDTA, 10 mM HEPES (pH 7.5), and 3× SSC. Filters were washed at 42°C in 1× SSC, 0.1% SDS twice for 30 min. The hybridization probe was the nick-translated kanamycin fragment of miniTn7Km. Nick-translation was performed according to standard procedures (Maniatis et al. 1982).

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