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Genes Dev. 1988 2: 137-149
Access the most recent version at doi:10.1101/gad.2.2.137
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]

Received October 22, 1987; revised version accepted December 21, 1987.

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 [Hermansteens 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 Griniter 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 1983; Ouartsi 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: miniTn7

<table>
<thead>
<tr>
<th>Tn7 element</th>
<th>Plasmid</th>
<th>Target plasmid*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn7</td>
<td>—</td>
<td>1.7 x 10^-3</td>
</tr>
<tr>
<td>miniTn7Cm5</td>
<td>pCW4</td>
<td>1.5 x 10^-4</td>
</tr>
<tr>
<td>miniTn7Cm6</td>
<td>pACYC184</td>
<td>&lt;2.2 x 10^-8</td>
</tr>
</tbody>
</table>

* 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.

As shown in Table 1, transposition of a Tn7-end element to both target plasmids is dependent on pCW4. 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 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 miniMu1 insertions reflects features of Mu transposition (Raibaud et al. 1979; Kamp and Kahmann 1981; M. Mizuuchi and K. Mizuuchi, pers. comm.).
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. The DNA was digested with EcoRI, size-separated by electrophoresis, and probed with the nick-translated kanamycin fragment of miniTn7Km. EcoRI digestion of pOX (59.4 kb in size) yields seven fragments ranging in size from 23.0 to 0.2 kb; there is no EcoRI cleavage site within miniTn7Km (1.8 kb in size). Therefore, this analysis is limited to determining the EcoRI 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 EcoRI 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 transformants 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 x 10^{-5} and 2.8 x 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 x 10^{-3}; to pOX-attTn7, 4.7 x 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 x 10^{-7} and <79.0 x 10^{-4} 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 x 10^{-7}), but transposition to pOX-attTn7 occurred at high, wild-type frequencies (average transposition frequency, 4.5 x 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-
Figure 2. miniMuΩ insertions in pCW4. The wide vertical box represents the Tn7 (open) and attTn7 (speckled) sequences in pCW4. Boldface numbers within the box indicate the length of Tn7 sequences in kb. Letters indicate sites cleaved by restriction enzymes. [B] BamHI, [Bg] BglII, [Bs] BstEII, [E] EcoRI, [H] HindIII, [Hp] HpaI, [PI] PvuI, [PII] PvuII. The narrow vertical box represents the pACYC184 sequences of pCW4. This region is drawn at two-thirds the scale of the Tn7 sequences. The total length of pACYC184 is 4.0 kb. The disrupted chloramphenicol acetyltransferase gene (CAT), the tetracycline resistance gene (Tc) and the origin of replication (ori) are also indicated. No restriction sites in pACYC184 sequences are shown. Ninety-six independent miniMuΩ insertions into pCW4 were obtained, as described in the text and in Materials and methods. Each insertion is identified by a number, and its map position is indicated by a horizontal line adjacent to its number. The positions of several insertions were indistinguishable by restriction enzyme analysis. The orientation of each insertion was also determined. (*) Mu left sequences are nearest attTn7; unmarked insertions are oriented with Mu right sequences nearest attTn7. The transposition phenotype of most of the pCW4 miniMuΩ plasmids was determined. Of insertions in pACYC184 sequences, only insertions 38, 16, and 42 were tested. Transposition of miniTnTCm from the chromosomal attTn7 site to pOX38-Gen and to pOX38-Gen, attTn7 was measured by a mating-out assay. The results were evaluated qualitatively for miniMuΩ insertions shown to the left of the vertical box and scores of -, +, or + + + were assigned. No score is shown for miniMuΩ insertions shown to the right of the vertical box. The transposition frequencies shown are the result of one, or are the average of two, independent assays. Both qualitative and quantitative results are shown for some insertions (e.g., all miniMuΩ insertions in attTn7). pCW4 does not promote miniMuΩ transposition (data not shown). (n.d.) Not determined.
position levels (average transposition frequency, 2.2 x 10^{-4}), but transposition to pOX was unaffected (average transposition frequency, 1.2 x 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 miniMuF 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 miniMuΩ 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 approximately 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 :: miniMuΩ plasmids could complement the transposition-defective Tn7 fragment deleted for this region. As shown in Figure 4A, pCW4 derivatives containing miniMuΩ insertions to the left of the 0.9-kb BglII site complemented the deletion, i.e., promoted transposition. Thus, the carboxyl terminus of tnsA lies between miniMuΩ insertions 93 [position 0.8 kb] and 19 [position 0.95 kb]. In other complementation tests, we found that the BglII 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 BglII site and miniMuΩ insertion 19. Complementation tests between various pCW4 :: miniMuΩ 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 miniMuΩ 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 miniMuΩ 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 miniMuΩ insertions 89 and 49 [Fig. 4B]. Smith and Jones (1986) sequenced the BamHI fragment in this region [see Fig. 4] and reported an ORF that starts immediately to the left of the 2.9-kb BamHI 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 miniMuΩ 39 [position 4.65 kb] and the 4.7-kb PvuII 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 :: miniMuΩ-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 :: miniMuΩ 72/pOX; (lanes 9-12) pCW4 tnsD :: miniMuΩ 72/pOX-attTn7; (lanes 13-16) pCW4 tnsE :: miniMuΩ 78/pOX-attTn7; (lane 17) pCW4/pOX-attTn7 as in lane 1 of Fig. 1.](image-url)
agreement with the coding capacity of their respective genes. Truncated polypeptides synthesized from various pCW4::tnsB :: miniMuI 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

<table>
<thead>
<tr>
<th>Table 2. tns fragment-promoted transposition</th>
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<tbody>
<tr>
<td><strong>Target plasmid</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>1. tnsABC</td>
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<td>2. tnsD</td>
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<tr>
<td></td>
</tr>
<tr>
<td>3. tnsE</td>
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<td></td>
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</tr>
<tr>
<td>4. tnsABC + tnsD</td>
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<td></td>
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</tr>
<tr>
<td>5. tnsABC + tnsE</td>
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<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* 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.

* 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 miniMufI 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::miniMufI mutation in *trans* (Table 3, line 3) but did complement a tnsA deletion [line 2]. This result suggests that the tnsA::miniMufI 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**

<table>
<thead>
<tr>
<th>pCW4 derivative</th>
<th>Complementing plasmid*</th>
<th>tnsA (pCW43)</th>
<th>tnsAB (pCW45)</th>
</tr>
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<tbody>
<tr>
<td>pCW4</td>
<td>None</td>
<td>4.4 x 10^-4</td>
<td>2.5 x 10^-5</td>
</tr>
<tr>
<td>ΔtnsA [pCW51]</td>
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<td>&lt;1.3 x 10^-8</td>
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<tr>
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<td>&lt;7.7 x 10^-8</td>
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<tr>
<td>tnsB::miniMufI 57</td>
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<td>&lt;6.9 x 10^-8</td>
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<tr>
<td>tnsB::miniMufI 11</td>
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<td>&lt;3.3 x 10^-8</td>
<td>n.d.</td>
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<tr>
<td>tnsB::miniMufI 89</td>
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<tr>
<td>tnsC::miniMufI 34</td>
<td></td>
<td>&lt;1.4 x 10^-7</td>
<td>n.d.</td>
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</table>

*Transposition frequencies of miniTn7Km from the chromosomal *attTn7* site to pOX were determined by a mating-out assay. tns plasmids are as indicated. pCW4::miniMufI 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 tnsB-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**

<table>
<thead>
<tr>
<th>tnsCD plasmid*</th>
<th>tnsABC* (pCW15)</th>
</tr>
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<td>tnsCD [pCW52]</td>
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<td>tnsCD [pCW53]</td>
<td>3.9 x 10^-1</td>
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<tr>
<td>tnsC::miniMufI 39 tnsD [pCW56]</td>
<td>1.1 x 10^-2</td>
</tr>
<tr>
<td>&lt;4.5 x 10^-8</td>
<td>-</td>
</tr>
</tbody>
</table>

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

*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::miniMufI mutation [line 3], as well as three different tnsB::miniMufI mutations [lines 4–6] and was unable to complement a tnsC::miniMufI 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::miniMufI* 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::miniMufI mutants are not polar on tnsD and, therefore, that tnsC and tnsD are separate transcription units. Each of the *tnsC::miniMufI* tnsD plasmids tested could complement the *tnsABC* plasmid and promote transposition to pOX-attTn7 (cf. lines 2, 3, and 5 to line 6). However, miniMufI 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. miniMufI 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 IncF1 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 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 miniMufl 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 diagramed in Figure 5. All five tns genes are transcribed in the same direction. Our analysis of polar effects of the tns :: miniMufl insertions is consistent with the hypothesis that

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

<table>
<thead>
<tr>
<th>tns plasmid</th>
<th>pOX</th>
<th>pOX-attTn7</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>b</em></td>
<td>1.7 x 10^-4</td>
<td>9.4 x 10^-3</td>
</tr>
<tr>
<td>tnsD</td>
<td>2.6 x 10^-5</td>
<td>2.2 x 10^-3</td>
</tr>
<tr>
<td>(pCW23)</td>
<td>4.2 x 10^-5</td>
<td>1.2 x 10^-3</td>
</tr>
<tr>
<td>(pCW25)</td>
<td>2.5 x 10^-5</td>
<td>1.6 x 10^-3</td>
</tr>
<tr>
<td>tnsE</td>
<td>5.5 x 10^-5</td>
<td>5.3 x 10^-3</td>
</tr>
<tr>
<td>(pCW30)</td>
<td>5.3 x 10^-5</td>
<td>1.6 x 10^-3</td>
</tr>
<tr>
<td>(pCW31)</td>
<td>4.7 x 10^-5</td>
<td>1.8 x 10^-3</td>
</tr>
<tr>
<td>(pCW32)</td>
<td>4.7 x 10^-5</td>
<td>1.8 x 10^-3</td>
</tr>
</tbody>
</table>

_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.)

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 transposition of the Tn7-encoded genes. dhfr encodes a dihydrofolate reductase [Fling and Richards 1983] that confers resistance to trimethoprim, and aadA encodes a 3'-5'-O-nucleotidytranferase [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 miniMufl 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 tnsE. 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 synopsis 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:

```
Tn7  tnsABC  tnsD  attTn7  tnsE  other sites
```

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

Strains

NLC51 is F- araD139Δ(araF-lac)U169 rpsL150 relA1 fliB5301 deoC1 ptsG25 tsrR valR recA56. 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 into a chromosomal 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 distinguishable from those of canonical Tn7 [Hauer and Shapiro 1984]. miniTn7/Km is Tn7S::Tn9ΔPstI [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; miniTn7/Km [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. miniTn7/Km 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 conjugal plasmid F and lacks y6 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 miniMuΩ 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-BglII fragment containing tnsA-tnsE was inserted into EcoRI-BamHI-digested pUC19.

pcW12. The EcoRI-BglII 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::miniMuΩ 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::miniMuΩ 39 containing tnsD was treated with Klenow fragment to repair the EcoRI end and was inserted into the HindIII 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 BsrEII 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 miniMuΩ insertions in pcW4 derivatives used in the formation of recombinant plasmids are indicated. The miniMuΩ 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] BglII, [Bs] BsrEII, [E] EcoRI, [H] HindIII, [Hp] HpaI, [P] PvuI, [Pil] PvuII.
the HindIII site of pACYC184, with the BsrEII site near vector Ptc (pCW31) or with the HpaI site near vector Ptc (pCW32).

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

pCW43. The EcoRI-BglII fragment from pCW4 :: miniMuΩ 57 containing the tnsA, tnsB junction and the BglII-HindIII fragment from pCW10 containing most of tnsA were inserted into EcoRI-HindIII-digested pUC19.

pCW45. The HindIII-HindIII fragment from pCW10 containing tnsA and tnsB was inserted into the HindIII site of pUC18, with tnsA near vector Plac.

cpCW5. The BglII-BglII fragment from pCW4 containing most of tnsA was deleted.

pCW52 and pCW53. The HpaI-HpaI fragment from pCW4 containing tnsC and tnsD was inserted into the HindIII site of pUC18, with tnsC near vector Plac (pCW52) or with tnsD near vector Plac (pCW53).

pCW54. The HpaI-HpaI fragment from pCW4 :: miniMuΩ 34 containing tnsC :: miniMuΩ 34 and tnsD was inserted into the HindIII site of pUC18, with tnsC :: miniMuΩ 34 near vector Plac.

cpCW55 and pCW56. The HpaI-HpaI fragment from pCW4 :: miniMuΩ 39 containing tnsC :: miniMuΩ 39 and tnsD was inserted into the HindIII 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 BamHI fragment from pH4501 [Prentki and Krisch 1984] is inserted at the BamHI 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 AatII, a restriction enzyme that cleaves pMK386 outside of the miniMuΩ sequences, to linearize cointegrate products, intramolecular restriction enzyme that cleaves pMK386 outside of the miniMuΩ. There is no AatII 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 TcRSp 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 simple single 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 PvuII and PvuI restriction analysis of plasmid DNA; orientation was determined by EcoRI or SphI 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 BamHI, BglII, BsrEII, HindIII, and HpaI. The positions of these sites within the Tn7 fragment in pCW4 are diagramed in Figures 2 and 6. There is no SphI site in the Tn7 fragment, and there are no BglII, HpaI, or PvuII 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, attTn7. 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 GenR RifR colonies. The number of exconjugants that had acquired a Tn7 derivative was determined by selection of TpR RifR, CamR RifR, or KmR RifR colonies for Tn7, miniTn7Cm, or miniTn7 Km 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 EcoRI, 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].

Acknowledgments

We are deeply grateful to Bob Craigie and Kiyoshi Mizuuchi for construction of miniMuΩ, in vitro mutagenesis of pCW4, and helpful discussions. We thank Pierre Prentki for pH4501, Nancy Kleckner for NK5830, Ken Kubo for help with the Southern analysis, other members of the Craig laboratory for useful discussions throughout this work, and Karyl Nakamura for excellent preparation of the manuscript. We also thank Mark Rogers and Dave Sherratt for useful discussions in naming the trans genes. Ira Herskowitz, Lorraine Marsh, and Susan Michaelis provided valuable discussions and comments on the manuscript. C.S.W. was supported by a National Science Foundation Fellowship. The work was supported by a grant from the National Institutes of Allergies and Infectious Diseases to N.L.C.

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