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Bacterial Transposon Tn7 Utilizes Two Different Classes of Target Sites

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Sites of transposon Tn7 insertion in the *Escherichia coli* chromosome were examined, and two distinct classes of target sites differing in nucleotide sequence were identified. The target site choice was found to be determined by Tn7-encoded transposition genes.

Most transposable elements move at low frequency and insert into many different target sites (5). The bacterial transposon Tn7 (2, 7; Fig. 1A) is unusual in that it can transpose at high frequency to a specific site in the *Escherichia coli* chromosome called *att*Tn7, located near the bacterial *glmS* gene (2, 10, 15, 16, 18; Fig. 1B). Tn7 can also transpose at low frequency to many different sites in plasmids (3, 4, 19). Tn7 insertion is accompanied by a 5-base-pair (bp) duplication of target sequences (10, 14, 16, 18, 20). Tn7 encodes five genes, *tnsABCDE*, that mediate two distinct but overlapping transposition pathways (23, 26; Fig. 1A). Previous studies have shown that *tnsABC+tnsD* promote transposition to *att*Tn7, whereas *tnsABC+tnsE* promote transposition to other sites.

We have analyzed Tn7 insertions in the *E. coli* chromosome; its large size provides a wider variety of target sites than do the plasmids used in previous studies. We report here that the target site sequences used by the *tnsD*-dependent (*tnsABC+tnsD*) and *tnsE*-dependent (*tnsABC+tnsE*) pathways differ dramatically. We show that the *tnsD*-dependent pathway has high target site selectivity, promoting transposition to *att*Tn7 and a limited number of other sites similar in sequence to *att*Tn7. By contrast, the *tnsE*-dependent pathway displays little target site selectivity, promoting transposition to many different sites not related in sequence to the *tnsD*-dependent sites.

We evaluated the frequency of Tn7 transposition to the *E. coli* chromosome (Table 1) by measuring the translocation of mini-Tn7Km, a Tn7 derivative containing a kanamycin resistance marker but lacking the *tns* genes (1, 18; Fig. 1C), from the replication- and integration-defective bacteriophage lambda derivative KK1 into bacteria containing the *tns* genes and selecting for kanamycin-resistant colonies (18). Strains and plasmids used are shown in Table 2. We also physically analyzed many of these mini-Tn7Km chromosomal insertions (Fig. 2 and Table 3). Genomic DNA was isolated (22), digested with restriction enzymes, resolved by agarose gel electrophoresis, and transferred to Nytran (Schleicher & Schuell, Inc.), and filters were hybridized with a nick-translated kanamycin fragment or a nick-translated Tn7R fragment.

tnsABC+tnsD promoted relatively high-frequency transposition to the chromosome (Table 1, line 4), and all such insertions were located in *att*Tn7 (Fig. 2A). To determine whether *tnsABC+tnsD* could also promote transposition to sites other than *att*Tn7, we used a slightly different target chromosome, one already containing a Tn7 derivative in *att*Tn7. We used this blocked *att*Tn7 chromosome because multiple Tn7 insertions in *att*Tn7 have not been observed (1, 12, 15). We detected *tnsD*-dependent transposition to the blocked *att*Tn7 chromosome at a frequency about 10^4-fold lower than to vacant *att*Tn7 (Table 1, line 4). Analysis of these *tnsD*-dependent insertions revealed that they occurred into a limited array of sites (Table 3), with a marked preference for sites DC-1, DC-2, and DC-3 (DC = *tnsABC+tnsD* chromosomal).

Comparison of the sequences of several DC sites with

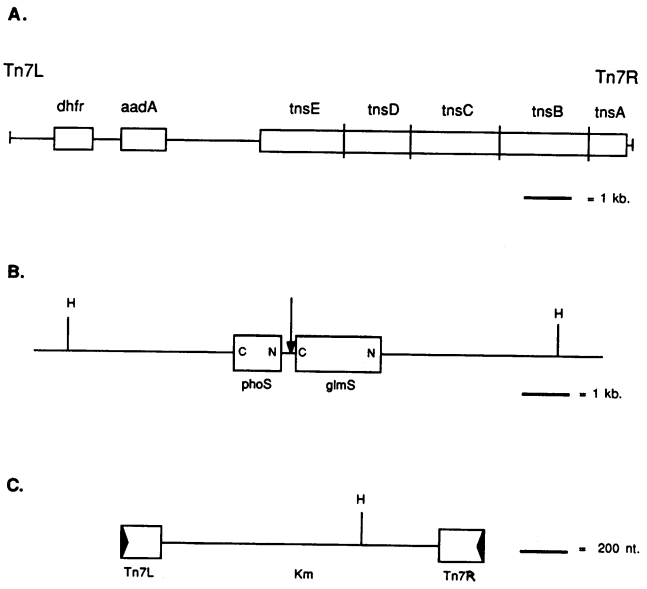

FIG. 1. Tn7, *att*Tn7, and mini-Tn7Km. (A) Tn7. The left (Tn7L) and right (Tn7R) ends of Tn7 are indicated. Tn7 contains two resistance determinants: *dhfr*, encoding a dihydrofolate reductase providing trimethoprim resistance, and *aadA*, encoding 3" (9)-*O*-nucleotidyltransferase providing spectinomycin and streptomycin resistance (8, 9, 25). The five Tn7 transposition genes *tnsABCDE* (23, 26) are shown. (B) Chromosomal *att*Tn7. The point of Tn7 insertion ↓ lies between the *phoS* and *glmS* genes, as previously described (10, 15, 16, 18). Tn7 insertion into *att*Tn7 is orientation specific such that Tn7L is proximal to *phoS* and Tn7R is proximal to *glmS* (15). (C) Mini-Tn7Km. The ends of Tn7 are shown as open boxes; the segment between them encodes a gene that confers resistance to kanamycin (1, 18). Restriction site: H, *Hin*dIII. kb, Kilobase; nt, nucleotides.

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TABLE 1. Transposition frequency to the *E. coli* chromosome[a]

tns gene	Plasmid	Mean transposition frequency (Km^r colonies/PFU) ± SEM (*n*)	
		Vacant *att*Tn*7*	*att*Tn*7*::mini-Tn*7*Cm
None	None	$<4.3 \times 10^{-8}$ (3)	$<6.3 \times 10^{-9}$ (3)
tnsABC	pCW21	$<4.3 \times 10^{-8}$ (3)	$<6.3 \times 10^{-9}$ (3)
tnsABCDE::mini-Mu	pCW4miniMuΩ76	ND[b]	$(1.0 \pm 0.3) \times 10^{-7}$ (6)
tnsABC+tnsD	pCW21 + pCW23	$(3.9 \pm 2.8) \times 10^{-4}$ (6)	$(3.0 \pm 1.1) \times 10^{-8}$ (3)
tnsABC+tnsE	pCW21 + pCW30	$(3.0 \pm 0.0) \times 10^{-7}$ (3)	$(9.1 \pm 2.4) \times 10^{-8}$ (3)
tnsABCDE	pCW4	$(8.0 \pm 1.0) \times 10^{-5}$ (3)	$(1.1 \pm 0.3) \times 10^{-7}$ (6)

[a] *E. coli* used were derivatives of NLC51 or LA214 containing the indicated plasmids (Table 1).
[b] ND, Not done.

each other and with *att*Tn*7* (Fig. 3) revealed considerable sequence similarity among all of these *tnsD*-dependent target sites. This similarity was most pronounced to one side (rightward) of the Tn*7* insertion points; little similarity was apparent at the actual insertion points. Strikingly, this region of similarity between the DC sites and *att*Tn*7* included a region that has been shown to be required for high-frequency, site-specific insertion into *att*Tn*7* (11, 18, 21). Because of their similarity to *att*Tn*7*, we call the DC sites pseudo-*att*Tn*7* sites. Thus, *tnsABC+tnsD* direct transposition to target sites of related nucleotide sequence, promoting high-frequency insertion in *att*Tn*7* and low-frequency insertion in pseudo-*att*Tn*7* sites.

tnsABC+tnsE promoted low-frequency transposition to the chromosome (Table 1, line 5). Physical analysis showed that the *tnsABC+tnsE* pathway ignored chromosomal *att*Tn*7* (compare Fig. 2A and B). A more detailed analysis of *tnsE*-dependent insertions in a blocked *att*Tn*7* chromosome demonstrated that EC (*tnsABC+tnsE* chromosomal) sites were located in many different positions (Table 3). Notably, no two *tnsE*-dependent insertions occurred at the same target site, nor were any *tnsE*-dependent insertions located in the identified pseudo-*att*Tn*7* sites.

To examine the Tn*7* insertions at higher resolution, we determined the nucleotide sequences for several DC and EC sites (Fig. 3). Chromosomal DNA segments containing mini-Tn*7*Km insertions were isolated by cloning into pUC18 with selection for kanamycin resistance (17). The sequences flanking the insertions were determined by the chain terminator method, using Sequenase (U.S. Biochemical Corp.), after alkali denaturation (24). Oligonucleotides specific to the left and right Tn*7* ends were used as primers.

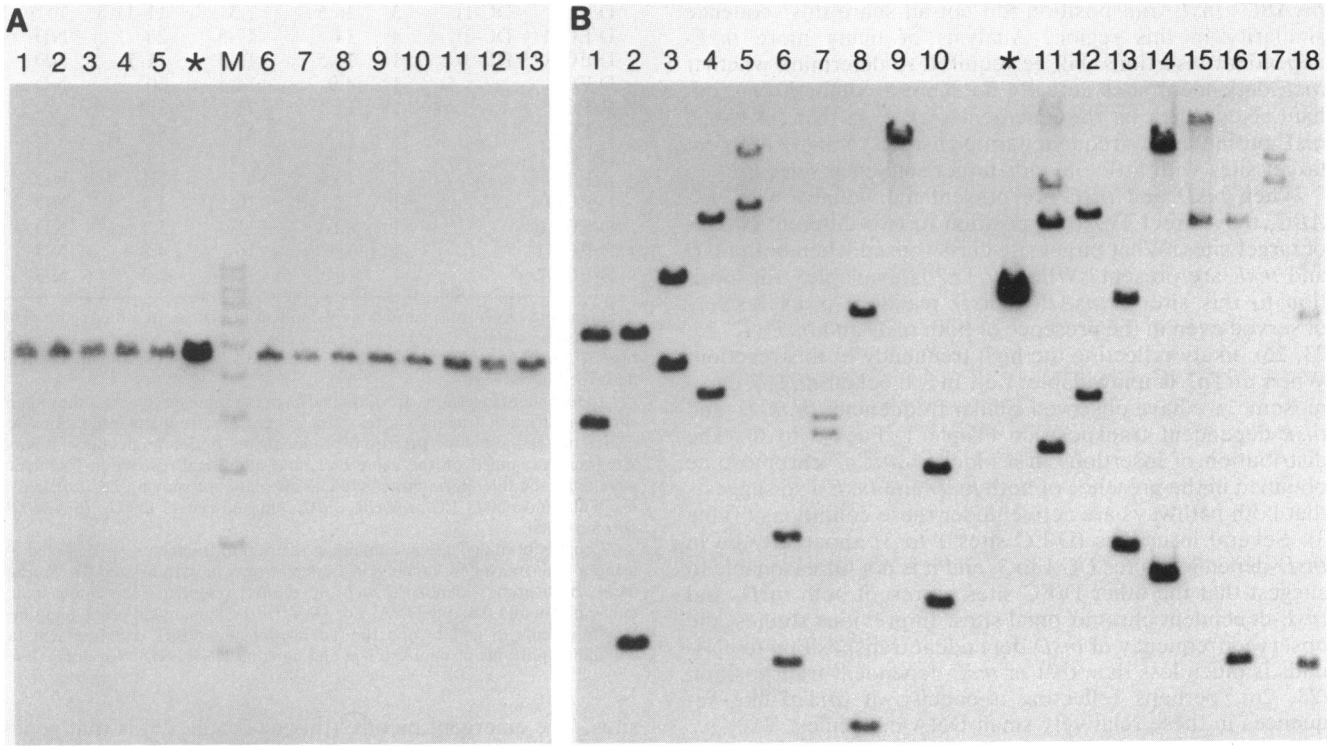

FIG. 2. Distribution of chromosomal insertion sites. Each numbered lane contains genomic DNA from a single transposition product that was physically analyzed as described in the text. Genomic DNA was digested with *Hin*dIII and hybridized with a nick-translated kanamycin fragment. The lanes marked * are from an *att*Tn*7*::mini-Tn*7*Km chromosome; digestion with *Hin*dIII generates two 6.5-kilobase fragments (Fig. 1) not resolved here which hybridize to the kanamycin probe. Lane M contains molecular weight markers. (A) *tnsABC+tnsD* insertions generated in a vacant *att*Tn*7* chromosome as in Table 1, line 4; (B) *tnsABC+tnsE* insertions in a vacant *att*Tn*7* chromosome generated as in Table 1, line 5.

TABLE 2. Strains and plasmids used

Strain or plasmid	Genotype or description	Reference
E. coli strains		
NLC51	F⁻ *araD139A* Δ(*argF-lac*)*U169* *rpsL150* *relA1* *flbB5301* *deoC1* *ptsF25* *rbsR* Val^r *recA56*	26
LA214	NLC51 *att*Tn*7*::mini-Tn*7*Cm[a]	1
Plasmids		
pCW4	pACYC184 derivative containing *tnsABCDE*	26
pCW4miniMuΩ76	Same as pCW4 except for insertion in *tnsE* inactivating that gene	26
pCW21	pACYC184 derivative containing *tnsABC*	26
pCW23	pUC18 derivative containing *tnsD*	26
pCW30	pUC18 derivative containing *tnsE*	26

[a] Mini-Tn*7*Cm is a Tn*7* derivative lacking the *tns* genes and containing a chloramphenicol resistance marker (1, 18).

Comparison of the DC sites with the EC sites (Fig. 3) did not reveal any striking sequence similarities between these two classes of target sites, although both resulted in a 5-bp target sequence duplication. A modest similarity among our EC sites was detectable about 15 bp leftward of the Tn*7* insertion point; however, its functional significance is unclear. We note that previously characterized plasmid insertions of Tn*7* (14, 16, 20), which likely resulted from *tnsABC+tnsE* transposition, do not all share this sequence similarity in this region. Analysis of many more *tnsE*-dependent insertions will be required to determine whether *tnsE*-dependent sites actually do share a common determinant. Nevertheless, the emergent picture is that *tnsABC+tnsE* promote low-frequency transposition to many different target sites with little obvious target sequence selectivity.

When *tnsD* and *tnsE* are present individually with *tnsABC*, they direct Tn*7* transposition to two different classes of target sites. What target site class is used when both *tnsD* and *tnsE* are present? When *att*Tn*7* is available, transposition to this site (a *tnsABC+tnsD* reaction) is exclusively observed even in the presence of both *tnsD* and *tnsE* (12, 15, 23, 26), likely reflecting the high frequency of this reaction. When *att*Tn*7* is unavailable, i.e., in a blocked *att*Tn*7* chromosome, we have observed similar frequencies of *tnsD*- and *tnsE*-dependent transposition (Table 1, lines 3 to 6). The distribution of insertions in a blocked *att*Tn*7* chromosome obtained in the presence of both *tnsD* and *tnsE* also suggests that both pathways are active under these conditions (Table 3). Several insertions (D/EC sites 1 to 3) apparently lie in *tnsD*-dependent sites DC 1 to 3, and it is not unreasonable to suggest that the other D/EC sites represent both *tnsD*- and *tnsE*-dependent chromosomal sites. In previous studies, the observed frequency of *tnsD*-dependent transposition to plasmids is much less than that of *tnsE*-dependent transposition (23, 26), perhaps reflecting a paucity of *att*Tn*7*-like sequences in these relatively small DNA molecules.

We have shown that Tn*7* can transpose to two distinct classes of target sites. *tnsABC+tnsD* transposition utilizes target sites of related sequence, directing insertion into *att*Tn*7* and pseudo-*att*Tn*7* sites. By contrast, *tnsABC+tnsE* transposition utilizes many different target sites not markedly related to each other or to the *tnsD*-dependent target sites. The emergent picture (this work; 23, 26) is that genes *tnsABC* provide functions common to all Tn*7* transposition events, whereas *tnsD* and *tnsE* determine the target site. An attractive hypothesis is that TnsD and TnsE are target DNA-binding proteins, TnsD being a specific DNA-binding protein recognizing *att*Tn*7* and pseudo-*att*Tn*7* sites and TnsE being a nonspecific DNA-binding protein. In support

TABLE 3. Physical analysis of mini-Tn*7*Km insertions in the blocked *att*Tn*7* chromosome[a]

Target site class[b]	No. of independent products	Restriction fragment size (kilobases)[c]			
		*Eco*RI	*Bam*HI	*Hin*dIII	
				a	b
tnsD					
DC-1	12	13.5–20	7.3–7.8	11–11.5	10.5
DC-2	10	11.5–15.5	15–17.5	18.5–20	7.1–8.0
DC-3	7	10.5–13	12–13.5	11–12	ND
DC-4	2	14	16.5–17	17–17.5	ND
DC-5	2	19	17	ND	ND
DC-6	1	19	6.5	9.0–9.8	3.4
DC-7	1	17	13.5	ND	ND
DC-8	1	12.5	12	10	ND
DC-9	1	11.5	20	18	ND
tnsE					
EC-1	1	7.3	17.5	3.0–3.1	2.1
EC-2	1	13	15	16	1.8–1.9
EC-3	1	4.4	17.5	16.5–19	8.1
EC-4	1	19	19	10.5–12.5	2.9
EC-5	1	9.3	15	16.5	4.2–4.6
EC-6	1	9.3	15	8.0	ND
EC-7	1	ND	16.5	9.0	ND
EC-8	1	11	15.5	17	ND
EC-9	1	7.7	17.5	18.5	8.0–8.7
EC-10	1	7.0	6.0	7.2	1.9–2.0
EC-11	1	13	15.5	5.4	2.4–2.6
EC-12	1	12	11.5	3.3	ND
EC-13	1	ND	15.5	6.1	1.8
EC-14	1	ND	15.5	5.8	2.3
EC-15	1	7.5	7.0	2.2–2.3	2.1
EC-16	1	8.0	17	3.3–3.5	2.9
tnsD+tnsE					
D/EC-1 (=DC-1)	3	16.5	7.5	11–11.5	10.5
D/EC-2 (=DC-2)	4	14	17.5	20	ND
D/EC-3 (=DC-3)	1	11.5	13.5	11.5	ND
D/EC-4	1	4.9	14.5	10	ND
D/EC-5	1	11.5	11	2.2	ND
D/EC-6	1	9.8	11	8.8	ND
D/EC-7	1	9.8	3.8	19.5	ND
D/EC-8	1	10.5	6.7	ND	ND
D/EC-9	1	15	10.5	3.4	ND
D/EC-10	1	8.6	11	5.1	ND
D/EC-11	1	7.3	16	4.8	ND
D/EC-12	1	18	14.5	2.3	ND

[a] Genomic DNA from various mini-Tn*7*Km insertions in a blocked *att*Tn*7* chromosome obtained as described in Table 1 (*tnsABC+tnsD* as in line 4; *tnsABC+tnsE* as in line 5; *tnsABCDE* as in line 6) was physically analyzed as described in the text.

[b] Independent insertions are classified as in the same target site if they share similar restriction fragment sizes; size ranges are from determinations repeated on separate blots. Insertions producing restriction fragments of related size were examined on the same blot, and additional restriction fragments were analyzed to confirm target site classes (data not shown). DC, *tnsABC+tnsD* chromosomal; EC, *tnsABC+tnsE* chromosomal; D/EC, *tnsABCDE* chromosomal.

[c] Lengths of mini-Tn*7*Km-containing restriction fragments, as detected by using a nick-translated kanamycin fragment or a nick-translated Tn*7*R fragment. Both probes identify *EcoRI* and *Bam*HI fragments containing intact mini-Tn*7*Km and flanking DNA. For *Hin*dIII, the kanamycin probe identifies two fragments (a and b) and the Tn*7*R probe identifies one fragment (a) containing portions of mini-Tn*7*Km and flanking DNA. ND, Not done.

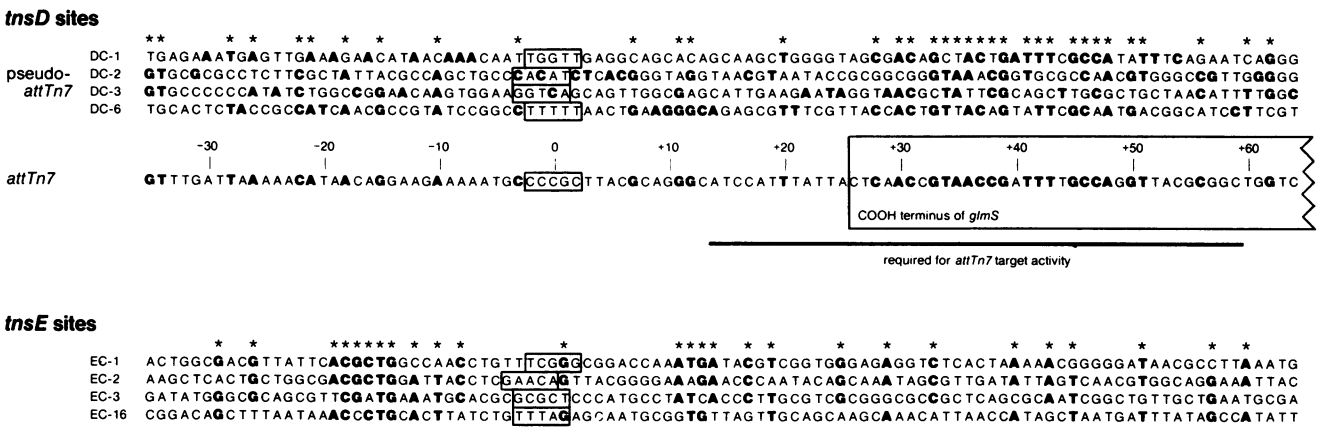

FIG. 3. Sequence analysis of Tn*7* insertion sites. The boxed nucleotides indicate the 5-bp chromosomal sequence duplicated upon Tn*7* insertion (10, 16, 18). The upper panel also shows the sequence of *att*Tn*7*; the central base pair of the duplicated sequence is designated 0; sequences leftward (towards *phoS*) are given negative values, and sequences rightward (towards *glmS*) are given positive values (see reference 18); the COOH terminus of the *glmS* gene product is also indicated (10). The bar underlines the region containing the nucleotides required for *att*Tn*7* target activity (11, 18, 21). Among the *tnsD*-dependent (DC) sites, sequence identities with *att*Tn*7* are indicated in boldface, and positions with three sequence identities among *att*Tn*7* and the pseudo-*att*Tn*7* sites are indicated with an asterisk. The sequence of two independent DC-1 insertions was determined; a difference of 1 bp in the positions of the duplicated nucleotides was observed (other duplication = GGTTG). A small degree of wobble has also been observed upon insertion in *att*Tn*7* (18). The lower panel shows the sequences of *tnsE*-dependent (EC) sites. Positions of sequence identity among at least three of the EC sites are indicated by boldface type and *. The chromosomal locations of the DC and EC sites are not known, although sequence comparisons suggest that the DC-3 insertion may lie within the bacterial *aceK* gene (6, 13). Nucleotide sequences of the DC and EC sites have been deposited in the GenBank data base (accession no. M31529 to M31536).

of this view, a *tnsD*-dependent binding activity that specifically recognizes *att*Tn*7* has been identified (27); the TnsD polypeptide copurifies with this *att*Tn*7*-binding activity (K. M. Kubo and N. L. Craig, unpublished observations). Moreover, the *tnsD*-dependent recognition region in *att*Tn*7* (between *att*Tn*7* +28 to +55) corresponds to the region of sequence similarity among the *tnsD* target sites, i.e., *att*Tn*7* and the pseudo-*att*Tn*7* sites.

Is the ability of Tn*7* to use two distinct classes of target sites advantageous? Target site-specific insertion into *att*Tn*7* provides a union between the host and Tn*7* in which no bacterial gene is inactivated, although sequences in a bacterial gene (*glmS*) are required for Tn*7* insertion (10). The ability of Tn*7* to transpose with little site selectivity is perhaps most useful in its transfer to and among plasmids.

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LITERATURE CITED

1. **Arciszewska, L. K., D. Drake, and N. L. Craig.** 1989. Transposon Tn7: cis-acting sequences in transposition and transposition immunity. J. Mol. Biol. **207**:35–52.
2. **Barth, P. T., N. Datta, R. W. Hedges, and N. J. Grinter.** 1976. Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. J. Bacteriol. **125**:800–810.
3. **Barth, P. T., and N. Grinter.** 1977. Map of plasmid RP4 derived by insertion of transposon C. J. Mol. Biol. **113**:455–474.
4. **Barth, P. T., N. J. Grinter, and D. E. Bradley.** 1978. Conjugal transfer system of plasmid RP4: analysis by transposon 7 insertion. J. Bacteriol. **133**:43–52.
5. **Berg, D. E., and M. M. Howe (ed.).** 1989. Mobile DNA. American Society for Microbiology, Washington, D.C.
6. **Cortay, J. C., F. Bleicher, C. Rieul, H. C. Reeves, and A. J. Cozzone.** 1988. Nucleotide sequence and expression of the *aceK* gene coding for isocitrate dehydrogenase kinase/phosphatase in *Escherichia coli*. J. Bacteriol. **170**:89–97.
7. **Craig, N. L.** 1989. Transposon Tn*7*, p. 211–225. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
8. **Fling, M., J. Kopf, and C. Richards.** 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3″ (9)-O-nucleotidyltransferase. Nucleic Acids Res. **13**:7095–7106.
9. **Fling, M., and C. Richards.** 1983. The nucleotide sequence of the trimethoprim-resistant dihydrofolate reductase gene harbored by Tn7. Nucleic Acids Res. **11**:5147–5158.
10. **Gay, N. J., V. L. J. Tybulewicz, and J. E. Walker.** 1986. Insertion of transposon Tn7 into the *Escherichia coli glmS* transcriptional terminator. Biochem J. **234**:111–117.
11. **Gringauz, E., K. A. Orle, C. S. Waddell, and N. L. Craig.** 1988. Recognition of *Escherichia coli att*Tn7 by transposon Tn7: lack of specific sequence requirements at the point of Tn7 insertion. J. Bacteriol. **170**:2832–2840.
12. **Hauer, B., and J. A. Shapiro.** 1984. Control of Tn7 transposition. Mol. Gen. Genet. **194**:149–158.
13. **Klumpp, D. J., D. W. Plank, L. J. Bowdin, C. S. Stueland, T. Chung, and D. C. LaPorte.** 1988. Nucleotide sequence of *aceK*, the gene encoding isocitrate dehydrogenase kinase/phosphatase. J. Bacteriol. **170**:2763–2769.
14. **Krishnapillai, V., M. Welxer, J. Nash, and D. H. Figurski.** 1987. Genetic basis of a Tn7 insertion mutation in the *trfA* region of the promiscuous IncP-1 plasmid R18 which affects its host range. Plasmid **17**:164–166.
15. **Lichenstein, C., and S. Brenner.** 1981. Site-specific properties of Tn7 transposition into the *E. coli* chromosome. Mol. Gen. Genet. **183**:380–387.
16. **Lichenstein, C., and S. Brenner.** 1982. Unique insertion site of Tn7 in the *E. coli* chromosome. Nature (London) **297**:601–603.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **McKown, R. L., K. A. Orle, T. Chen, and N. L. Craig.** 1988.

Sequence requirements of *Escherichia coli att*Tn*7*, a specific site of transposon Tn*7* insertion. J. Bacteriol. **170**:352–358.
19. **Moore, R. J., and V. Krishnapillai.** 1982. Tn7 and Tn501 insertions into the *Pseudomonas aeruginosa* plasmid R91-5: mapping of two transfer regions. J. Bacteriol. **149**:276–283.
20. **Nash, J., and V. Krishnapillai.** 1987. DNA sequence analysis of host range mutants of the promiscuous IncP-1 plasmids R18 and R68 with Tn7 insertions in *oriV*. Plasmid **18**:35–45.
21. **Qadri, M. I., C. C. Flores, A. J. Davis, and C. P. Lichenstein.** 1989. Genetic analysis of *attTn7*, the transposon Tn7 attachment site in *Escherichia coli*, using a novel M13-based transduction assay. J. Mol. Biol. **207**:85–98.
22. **Raleigh, E. A., and N. Kleckner.** 1984. Multiple IS10 rearrangements in *E. coli*. J. Mol. Biol. **173**:437–461.
23. **Rogers, M., N. Ekaterinaki, E. Nimmo, and D. Sherratt.** 1986. Analysis of Tn7 transposition. Mol. Gen. Genet. **205**:550–556.
24. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
25. **Simonsen, C. C., E. Y. Chen, and A. D. Levinson.** 1983. Identification of the type 1 trimethoprim-resistant dihydrofolate reductase specified by the *Escherichia coli* R-plasmid R483: comparison with procaryotic and eucaryotic dihydrofolate reductases. J. Bacteriol. **155**:1001–1008.
26. **Waddell, C. S., and N. L. Craig.** 1988. Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes. Dev. **2**:137–149.
27. **Waddell, C. S., and N. L. Craig.** 1989. Tn7 transposition: recognition of the *attTn7* target sequence. Proc. Natl. Acad. Sci. USA **86**:3958–3962.