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A Gene and Its Product Required for Transposition of Resistance Transposon Tn*2603*

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Tn*2603* is a multiple-resistance transposon encoding resistance to ampicillin, streptomycin, sulfonamide, and mercury and having a molecular size of 20 kilobase pairs, with 200-base-pair inverted repeats at both ends. The essential sites and functions of Tn*2603* which are required for its transposition were determined through construction and characterization of various deletion mutants affecting the efficiency of transposition. Deletions were introduced in plasmid pMK1::Tn*2603* by partial digestion with restriction endonuclease *Eco*RI in vitro. Analysis of deletion mutants showed that the inverted repeat segments at both ends and the *trans*-acting diffusible product(s) encoded in the right-hand side of the central portion were required for the transposition of Tn*2603*. An essential gene product was revealed as a protein having a molecular weight of 110,000 by analysis of polypeptides synthesized in *Escherichia coli* minicells. This protein was assumed to be the so-called transposase.

Tn*2603* is 20 kilobases (kb) in length and encodes multiple resistance to ampicillin, streptomycin, sulfonamide, and mercury (19). Ampicillin resistance of Tn*2603* is due to production of a type II β-lactamase (alternatively, oxacillin-hydrolyzing β-lactamase, designated OXA-1 [17]). Tn*2603* is the only movable element capable of transposing the gene for the type II enzyme known at present (9). In view of evidence showing that the type II enzyme gene has, in most instances, been recognized together with streptomycin, sulfonamide, and mercury resistance genes on naturally occurring plasmids, it can be speculated that a multiple-resistance transposon such as Tn*2603* plays a major role in the distribution of the type II β-lactamase gene in nature (18). This gene is distinct from the type I β-lactamase gene whose transposition is mediated by a transposon specifying only ampicillin resistance, for example, Tn*3* (10). Recently, we demonstrated that Tn*2603* is closely related to two other multiple-resistance transposons, Tn*21* and Tn*4*, suggesting that these transposons have been generated from a common ancestor (18).

Recently, evidence has contributed to our understanding of the mechanism of transposition, and models of the molecular mechanism have been postulated (1, 2, 5, 15). These models involve two stages. The first step is the formation of a cointegrate structure of two replicons joined by two copies of the transposon. In Tn*3*, the cointegrate formation requires the *tnpA* gene product, the transposase, specified by Tn*3* itself (8). The second step is resolution of the cointegrate structure resulting in a copy of the transposon at its original site in the donor replicon and a new copy in the target replicon. Resolution is mediated by the *tnpR* gene product, a resolvase (12, 13). A transposase gene required for transposition has also been defined in other transposons, e.g., Tn*5* (14) and Tn*10* (4).

To determine the gene(s) of Tn*2603* needed for transposition, we constructed and characterized a series of deletion mutants in the transposon. We also detected polypeptides specified by Tn*2603* in an *Escherichia coli* minicell system and demonstrated the potential transposase polypeptide of Tn*2603*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used were *E. coli* K-12 C600 (*thr leu thi lac tonA supE*), AB2463 (*recA thr leu thi lac gal ara xyl mtl pro his arg rpsL tsx sup-37*), WA5023 Rif^r, a rifampin-resistant (Rif^r) mutant of WA5023 (*polA thy*), and χ2207 (*minA minB nal*). Strains WA5023 and χ2207 were kindly provided by M. Yoshikawa and M. Inoue, respectively. The plasmids pMK1::Tn*2603*-1, pMB9::Tn*2603*-1, R386, and pACYC184 were previously described (18, 19). pMK1::Tn*2603*-11 is a plasmid with Tn*2603* from R388::Tn*2603*-1 inserted into pMK1 (19). pMK1 is a variant of plasmid ColE1, contains Tn*5*, and has a molecular size of 12 kb.

In vitro formation of deletions in pMK1::Tn*2603*-1 DNA by digestion with *Eco*RI endonuclease. A 1-μg amount of pMK1::Tn*2603*-1 DNA was partially digested with 1 U of *Eco*RI endonuclease for 15 min at 37°C. After inactivation of *Eco*RI endonuclease by heating for 15 min at 65°C, digested DNA was ligated with T4 ligase (Takara Shuzo Co., Ltd., Kyoto, Japan) and transformed into competent *E. coli* C600 cells prepared by the method of Cohen et al. (3). Ampicillin-

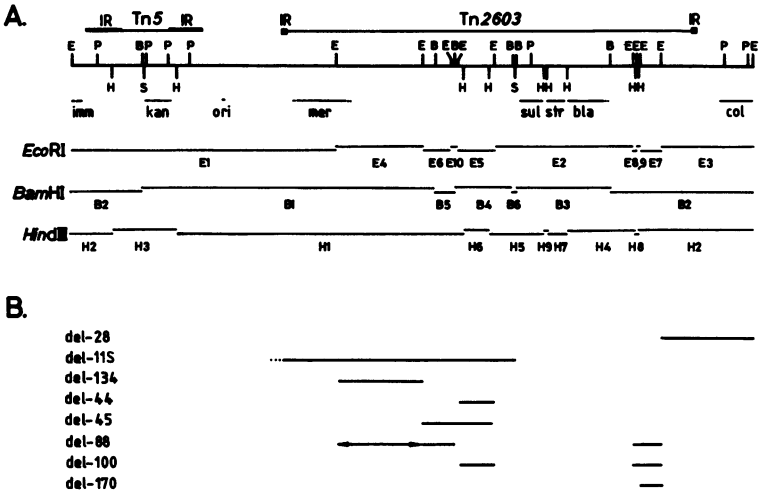

FIG. 1. Restriction cleavage map of pMK1::Tn*2603*-1 (A) and locations of deletions in deletion mutants (B). Abbreviations: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; S, *Sal*I; P, *Pst*I; imm, immunity to colicin E1; kan, kanamycin; ori, origin of replication; mer, mercury; sul, sulfonamide; str, streptomycin; bla, β-lactamase; col, colicin E1. The designations of fragments are indicated under the cleavage map in (A). Notations in (B): (—), deleted region; (↔), inverted region.

and kanamycin-resistant transformants were selected and then examined for resistance to streptomycin, sulfonamide, and mercury chloride (Hg) and colicin production. In the transformants resistant to ampicillin, streptomycin, sulfonamide, and kanamycin, colicin production or nonproduction was expected to be due to derivatives retaining the intact inverted repeat segments at both ends or to derivatives deleted for the right inverted repeat segment of Tn*2603*, respectively. Deleted molecules were initially monitored by agarose gel electrophoresis of colony lysates from appropriate clones and then verified by a restriction endonuclease digestion analysis of the purified plasmid DNA, using various endonucleases.

Polypeptide synthesis in minicells. Minicell-producing strain χ2207 was transformed with plasmid DNA. Isolation of minicells, labeling of polypeptides with ^{14}C-amino acids (45 mCi/mmol; Commissariat à L'Energie Atomique, Paris, France), and lysis of minicells were carried out by the methods of Veltkamp et al. (16). Minicell lysates were loaded on sodium dodecyl sulfate-polyacrylamide gels as described by Laemmli (11). After electrophoresis for 16 h at 12 mA, gels were stained with Coomassie brilliant blue R-250, destained, and soaked with En^{3}Hance (New England Nuclear Corp., Boston, Mass.). Gels were dried by heating at 65°C, and fluorography was carried out for 8 days at −80°C.

Determination of Tn*2603*-promoted transposition by a conjugal mating assay. Determination of transposition frequency was carried out essentially by the method described before (19), except that WA5023 Rif^r was used instead of P3478 Rif^r as the recipient bacterium.

Other methods. Plasmid DNA preparation, restriction endonuclease digestion, agarose gel electrophoresis, ligation, and transformation were done by the methods previously described (18).

RESULTS

Construction of deletions in pMK1::Tn*2603* plasmids. pMK1::Tn*2603*-1 is a derivative of plasmid pMK1 with Tn*2603* inserted in the ColE1 plasmid region and has a molecular size of 33 kb (19). A restriction cleavage map of the plasmid has been constructed with various restriction endonucleases (Fig. 1A). To make deletions which span the entire length of Tn*2603*, except the segment for ampicillin, streptomycin, and sulfonamide resistance, we used *Eco*RI endonuclease digestion since there are cleavage sites for this enzyme scattered throughout the transposon. Plasmid pMK1::Tn*2603*-1 DNA was partially digested with *Eco*RI endonuclease, ligated, and transformed into *E. coli* C600. Resultant transformants were examined for their resistance determinants and colicin production (Table 1). Plasmid DNA was isolated from each transformant and digested with *Eco*RI endonuclease to determine the deleted fragment(s). Agarose gel electrophoresis patterns of a representative set of deletion mutants isolated are shown in Fig. 2. Mutant del-11S (Fig. 2, slot j) was generated from pMK1::Tn*2603*-11 by digestion with *Sal*I endonuclease. pMK1::Tn*2603*-11 is a plasmid with Tn*2603* inserted into one of the two inverted repeats of Tn*5* on pMK1 in an opposite orientation with respect to pMK1::Tn*2603*-1 (unpublished data). These deletion derivatives were examined by cleavage analysis with other endonucleases to determine whether the remaining *Eco*RI fragments maintained their original order (Fig. 1B). In all dele-

TABLE 1. Relative transposition frequencies and complementation analysis

Plasmid	Relevant markers[a]	Colicin production	Relative transposition frequencies	Relative frequencies complemented with fragment:	
				B2	H2
pMK1::Tn*2603*-1	Ap^r, Sm^r, Su^r, Hg^r, Km^r	+	1.0[b]	—[c]	—
del-28	Ap^r, Sm^r, Su^r, Hg^r, Km^r	−	<0.01	<0.01	<0.01
del-11S	Ap^r, Sm^r, Su^r, Hg^s, Km^r	+	<0.01	<0.01	<0.01
del-134	Ap^r, Sm^r, Su^r, Hg^s, Km^r	+	0.29	—	—
del-44	Ap^r, Sm^r, Su^r, Hg^r, Km^r	+	1.1	—	—
del-45	Ap^r, Sm^r, Su^r, Hg^r, Km^r	+	1.9	—	—
del-88	Ap^r, Sm^r, Su^r, Hg^s, Km^r	+	<0.01	—	—
del-100	Ap^r, Sm^r, Su^r, Hg^r, Km^r	+	<0.01	—	—
del-170	Ap^r, Sm^r, Su^r, Hg^r, Km^r	+	<0.01	0.14	0.13

[a] Ap, Ampicillin; Sm, streptomycin; Su, sulfonamide; Km, kanamycin.
[b] Transposition frequency was 4.8×10^{-3} in this experiment.
[c] —, Not tested.

tion derivatives except the del-88 plasmid, the fragments were retained in an arrangement similar to that of fragments of the parent plasmid pMK1::Tn*2603*-1. In the del-88 plasmid, the E4 fragment was inverted at the original position, resulting in inactivation of the Hg resistance gene.

Characterization of transposition proficiency of deletion mutants. To test the ability of the deletion mutants to transpose, we determined transposition frequencies by a conjugal mating assay as follows. Plasmid DNA of parent or each deletion mutant was transformed into *E. coli recA* strain AB2463 harboring the transferrable plasmid R386. Resultant transformants were mated with *E. coli polA* strain WA5023 Rif^r, and the R386-containing transconjugants were selected by screening for tetracycline and rifampin resistance, whereas the R386::Tn transconjugants were assayed by screening for ampicillin, tetracycline, and rifampin resistance. The frequency of transposition into R386-containing transconjugants is presented as a relative value compared with that of parental Tn*2603*-promoted transposition (Table 1). del-28 and del-11S, which have lost right and left inverted repeat segments, respectively, could not transpose. These results confirmed that the inverted repeat segments at both ends of Tn*2603* are necessary for its transposition, as has been shown for other known transposons (4, 7, 14). del-134, del-44, and del-45, which have lost regions from the center to the left of the element, transposed at a level close to that of parental Tn*2603*. On the other hand, del-88, del-100, and del-170 could not transpose at all. The deleted region common to these mutants was the E7 fragment. These observations suggested that one or more essential functions are encoded in the right-hand region of Tn*2603*, encompassing the E7 fragment.

To determine whether the essential function encoded in the deleted region of the transposition-deficient mutants is the gene product or a site required for transposition, we attempted a complementation analysis for transposition.

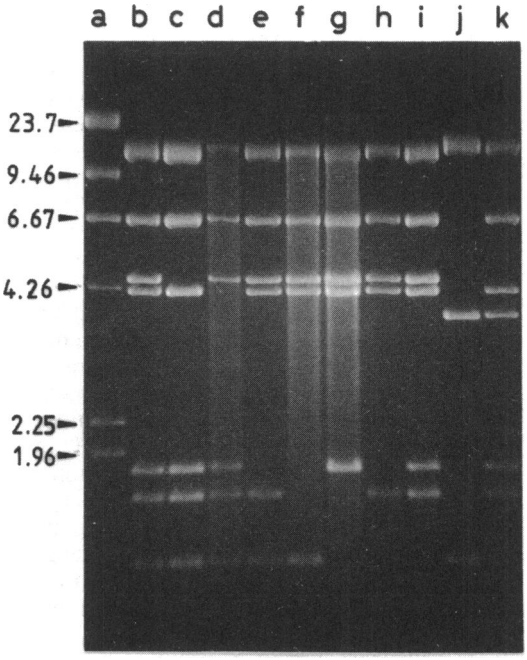

FIG. 2. Agarose gel electrophoresis of *Eco*RI digests of pMK1::Tn*2603*-1 and pMK1::Tn*2603*-11 and their deletion derivatives. Lane a, Bacteriophage λ DNA digested with *Hin*dIII endonuclease for molecular size standards (molecular sizes of the fragments are indicated in kb on the left side). *Eco*RI digestion patterns of: lane b, pMK1::Tn*2603*-1; lane c, del-28; lane d, del-134; lane e, del-44; lane f, del-45; lane g, del-88; lane h, del-100; lane i, del-170; lane j, del-11S; lane k, pMK1::Tn*2603*-11.

pACYC184 derivatives, in which a B2 or H2 fragment contained the E7 region indicated in Fig. 1, were constructed and then transformed into *E. coli* AB2463 harboring R386 and the transposition-deficient mutants, such as del-28, del-11S, and del-170. If this region encodes the gene product(s) required for transposition, these mutants would be expected to be complemented in *trans* by the coexisting plasmid. The ability of del-170 to transpose was demonstrably complemented by plasmids carrying the H2 (13%) or B2 (14%) fragment relative to the wild-type plasmid. These observations suggested that the right-hand region encompassing the E7 fragment in the element encodes at least one gene product necessary for transposition of Tn*2603*. However, the abilities of del-28 and del-11S to transpose were not complemented, suggesting that the inverted repeats are *cis*-acting structures necessary for transposition.

Tn*2603*-specific polypeptides produced in *E. coli* minicells. We next attempted to identify the polypeptides encoded by Tn*2603*, especially those involved in its transposition, by using an *E. coli* minicell system. Minicell-producing strain χ2207 was transformed with a plasmid pACYC184 derivative which contains H2 and B2 fragments from pMK1::Tn*2603*-1, and the polypeptides produced in the resultant clones were analyzed (Fig. 3A). After fluorography, the X-ray film was scanned to increase the sensitivity; the regions covering the high-molecular-weight polypeptides are shown in Fig. 3B. The B2 fragment contains, in addition to Tn*2603* genes, the colicin E1 structural gene and the inverted repeat segment of Tn*5* that specifically codes for the transposase (14), whereas the H2 fragment contains the colicin E1 gene and a part of the same inverted repeat. The polypeptides assigned to the colicin E1 gene and the transposase encoded by the inverted repeat segment of Tn*5* (14) are shown in Fig. 3. A 45,000-molecular-weight polypeptide of pACYC184-H2 was assigned to a truncated polypeptide of transposase encoded by Tn*5*. The results revealed that both H2 and B2 fragments specify a polypeptide having a molecular weight of 110,000. The gene encoding the polypeptide was found in the region of 3.1 kb between the right inverted repeat segment and the nearest *Hin*dIII site of Tn*2603*. This 3.1-kb DNA region is of a size that could account for the total coding capacity of the 110,000-molecular-weight polypeptide. The deletion of the E7 fragment (1.2 kb) in the del-170 mutant will therefore lead to the loss of the intact 110,000-molecular-weight polypeptide. The 110,000-molecular-weight polypeptide is implicated as a transposase of Tn*2603* because pACYC184-H2 and pACYC184-B2 complemented in *trans* the transposition deficiency of del-170. To compare each transposase of Tn*2603* with an ampicillin resistance transposon, Tn*2602*, the polypeptides from plasmid pTY2, a *tnpA*$^+$ *tnpR*$^-$ *bla*$^-$ mutant constructed from pMK1::Tn*2602*-1 (20) by *Bam*HI digestion and ligation, were electrophoresed on the same gel. The 110,000-molecular-weight polypeptide had a molecular weight identical to that of a transposase of Tn*2602* (Fig. 3, slot e). Furthermore, a 21,000-molecular-weight polypeptide was produced by B2 but not by H2. A function of this polypeptide will be discussed.

DISCUSSION

In this paper, we have demonstrated the essential regions of Tn*2603* required for its own transposition by genetic and functional analysis of various deletion mutants. Moreover, we determined the gene product encoded by one of the regions by an analysis of the polypeptides synthesized in minicells.

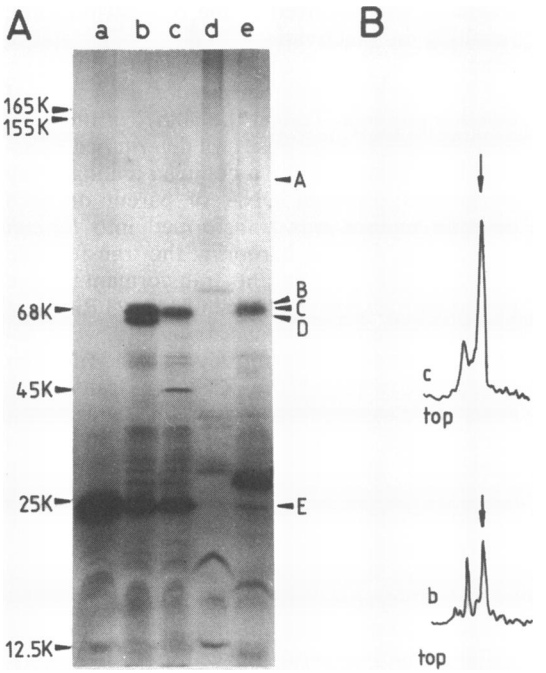

FIG. 3. Polypeptides synthesized in minicells fractionated by 10 to 15% sodium dodecyl sulfate-polyacrylamide gradient gel. (A) Fluorography of the gel. Slot a, pACYC184; slot b, pACYC184-B2; slot c, pACYC184-H2; slot d, pMB9::Tn*2603*; slot e, pTY2. Marker A, Transposase of Tn*2602*; marker C, colicin E1; markers B and D, transposase of Tn*5*; marker E, chloramphenicol acetyltransferase. Standard molecular weights × 10^3 (K) are indicated on the left side of the gel. (B) Scanning of the region fractionating the transposase of Tn*2603* in lanes b and c. Arrows indicate the transposase of Tn*2603*.

The right-hand side of the central portion of Tn*2603* (Fig. 1) encoded at least one function which is a *trans*-acting, diffusible product required for transposition. The gene product was defined as a polypeptide having a molecular weight of 110,000. Tn*2603* has inverted repeat sequences of about 200 base pairs at both ends, as previously determined by electron microscopic heteroduplex analysis but not precisely by sequencing (19). Deletions including the terminal inverted repeat sequences cannot transpose under any circumstances, whereas deletions in the internal region of Tn*2603* appear capable of transposition by complementation with the corresponding region from the element.

The notion that transposons specify functions involved in their own transposition was first proposed for Tn*3* by Heffron et al. (7). Their studies, in which deletion mutants affecting the transposability of Tn*3* were used, indicated that its gene product, thereafter called transposase, and the terminal inverted repeat sequences are involved in normal Tn*3* transposition. The transposase was specified in the left-hand side of the central region, although the intact terminal inverted repeat structures were required for transposition. A similar analysis has been reported for Tn*5* and Tn*10* (4, 14). In both elements, the respective inverted repeat segments, only one of the pairs in each case, contain all of the transposon-coded information required for normal transposition. Tn*5* and Tn*10* can therefore be thought of as being composite elements which include structurally intact insertion sequence-like elements. In Tn*2603*, the potential transposase was not specified in the terminal inverted repeat segments but in the central portion, as shown for the class of elements represented by Tn*3*. Tn*2603*-encoded potential transposase has a molecular weight very similar to that of the transposase specified by Tn*3* (Fig. 3). This is an interesting observation in consideration of the divergency or universality of transposon-specific enzymes which are involved in transposition.

The coding region of the potential transposase of Tn*2603* was located in a region of 3.1 kb between the right inverted repeat segment and the nearest *Hin*dIII cleavage site in the element. We also identified a polypeptide which had a molecular weight of 21,000 encoded by the region adjacent to the left of the coding region of the 110,000-molecular-weight potential transposase. This finding can be correlated with the observation that Tn*3* and γδ specify 21,000- or 19,500-molecular-weight proteins from genes located adjacent to the transposase genes (6, 13). The proteins are also involved in the regulation of expression of the transposase gene and the resolution of the intermediate cointegrate (1, 13). Recently, we have shown that the 21,000-molecular-weight polypeptide of Tn*2603* resolves the cointegrate as an intermediate of its transposition. Furthermore, the loss of the intact polypeptide did not lead to an increase in the frequency of formation of the cointegrate relative to that of the transposition of wild type, suggesting that the polypeptide does not repress or weaken the expression of the transposase gene (M. Tanaka et al., submitted for publication). This observation is possibly associated with the result that the transposase of Tn*2603* was produced by the B2 fragment producing the 21,000-molecular-weight polypeptide, although the fourfold-higher production of transposase was observed by the H2 fragment missing the 21,000-molecular-weight polypeptide (Fig. 3B), as distinct from Tn*3*, whose product has the functions of repressor and resolvase (1, 6, 13).

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LITERATURE CITED

1. **Arthur, A., and D. Sherratt.** 1979. Dissection of the transposition process: a transposon-encoded site-specific recombination system. Mol. Gen. Genet. **176**:267–274.
2. **Bukhari, A. I.** 1981. Models of DNA transposition. Trends Biochem. Sci. **6**:56–60.
3. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
4. **Foster, T. J., M. A. Davis, D. E. Roberts, K. Takeshita, and N. Kleckner.** 1981. Genetic organization of transposon Tn*10*. Cell **23**:201–213.
5. **Galas, D. J., and M. Chandler.** 1981. On the molecular mechanism of transposition. Proc. Natl. Acad. Sci. U.S.A. **78**:4858–4862.
6. **Gill, R. E., F. Heffron, and S. Falkow.** 1979. Identification of the protein encoded by the transposable element Tn*3* which is required for its transposition. Nature (London) **282**:797–801.
7. **Heffron, F., P. Bedinger, J. J. Champoux, and S. Falkow.** 1977. Deletions affecting the transposition of an antibiotic resistance gene. Proc. Natl. Acad. Sci. U.S.A. **69**:702–706.
8. **Heffron, F., B. J. McCarthy, H. Ohtsubo, and E. Ohtsubo.** 1979. DNA sequence analysis of the transposon Tn*3*: three genes and three sites involved in transposition of Tn*3*. Cell **18**:1153–1163.
9. **Kopecko, D. J.** 1980. Specialized genetic recombination systems in bacteria: their involvement in gene expression and evolution. Prog. Mol. Subcell. Biol. **7**:135–234.
10. **Kopecko, D. J., J. Brevet, and S. N. Cohen.** 1976. Involvement of multiple translocating DNA segments and recombinational hot spots in the structural evolution of bacterial plasmids. J. Mol. Biol. **108**:333–360.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **McCormick, M., W. Wishart, H. Ohtsubo, F. Heffron, and E. Ohtsubo.** 1981. Plasmid cointegration and their resolution mediated by transposon Tn*3* mutants. Gene **15**:103–118.
13. **Reed, R. R.** 1981. Resolution of cointegrates between transposons γδ and Tn*3* defines the recombination site. Proc. Natl. Acad. Sci. U.S.A. **78**:3428–3432.

14. **Rothstein, S. J., R. A. Jorgensen, K. Postle, and W. S. Reznikoff.** 1980. The inverted repeats of Tn*5* are functionally different. Cell **19**:795–805.
15. **Shapiro, J. A.** 1979. Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Natl. Acad. Sci. U.S.A. **76**:1933–1937.
16. **Veltkamp, E., W. Barendsen, and H. J. Nijkamp.** 1974. Influence of protein and ribonucleic acid synthesis on the replication of the bacteriocinogenic factor Clo DF13 in *Escherichia coli* cells and minicells. J. Bacteriol. **118**:165–174.
17. **Yamagishi, S., K. Ohara, T. Sawai, and S. Mitsuhashi.** 1969. The purification and properties of penicillin β-lactamases mediated by transmissible R factor in *Escherichia coli*. J. Biochem. **66**:11–20.
18. **Yamamoto, T., M. Tanaka, R. Baba, and S. Yamagishi.** 1981. Physical and functional mapping of Tn*2603*, a transposon encoding ampicillin, streptomycin, sulfonamide, and mercury resistance. Mol. Gen. Genet. **181**:464–469.
19. **Yamamoto, T., M. Tanaka, C. Nohara, Y. Fukunaga, and S. Yamagishi.** 1981. Transposition of the oxacillin-hydrolyzing penicillinase gene. J. Bacteriol. **145**:808–813.
20. **Yamamoto, T., S. Yamagata, K. Horii, and S. Yamagishi.** 1982. Comparison of transcription of β-lactamase genes specified by various ampicillin transposons. J. Bacteriol. **150**:269–276.