JOURNAL OF BACTERIOLOGY, July 1988, p. 3046–3052
0021-9193/88/073046-07$02.00/0
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Vol. 170, No. 7

# Sequence Analysis of Termini of Conjugative Transposon Tn*916*

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Received 28 December 1987/Accepted 11 April 1988

**Transposon Tn*916* is a 16.4-kilobase, broad-host-range, conjugative transposon originally identified on the chromosome of *Enterococcus* (*Streptococcus*) *faecalis* DS16. Its termini have been sequenced along with the junction regions for two different insertions. The ends were found to contain imperfect inverted repeat sequences with identity at 20 of 26 nucleotides. Further in from the ends, imperfect directly repeated sequences were present, with 24 of 27 nucleotides matching. The transposon junction regions contained homologous segments but of a nature not consistent with a direct duplication of the target sequence. Within the right terminus was a potential outwardly reading promoter. Tn*916* is believed to transpose via an excision-insertion mechanism; based on the analyses of the termini, as well as two target sequences (before insertion and after excision), a possible model is suggested.**

Transposon Tn*916* is a conjugative transposon originally identified on the chromosome of *Enterococcus* (*Streptococcus*) *faecalis* DS16 (16). It is the prototype of a closely related family of transposons that includes Tn*918* (8), Tn*920* (32a; D. B. Clewell, F. Y. An, and B. E. Murray, *in* R. Lutticken, ed., *Proceedings of the Xth Lancefield Symposium*, in press), and Tn*925* (7) from *E. faecalis* and Tn*919* (15) from *Streptococcus sanguis*. These elements all encode tetracycline resistance and have a size range of 16 to 23 kilobases (kb). Similar transposons with multiple drug resistance determinants have been identified on the chromosomes of various streptococcal species (e.g., Tn*1545* from *Streptococcus pneumoniae* [11, 12; also see reference 39]; Tn*3951* from *Streptococcus agalactiae* [22, 36, 38]; and Tn*3701* from *Streptococcus pyogenes* [27; T. Horaud, personal communication]), and such elements are as large as 60 kb. An interesting property of almost all conjugative transposons is their ability to insert into *E. faecalis* hemolysin plasmids (e.g., pAD1 [10, 16]) so as to give rise to an enhanced expression of hemolysin. For a recent review of conjugative transposons, see Clewell and Gawron-Burke (9).

Tn*916* has been cloned in *Escherichia coli* on plasmid vectors, where it has been mapped and studied genetically by using Tn*5* as an insertional mutagen (35, 41). In the case of certain plasmid vectors, Tn*916*-containing chimeras are unstable under nonselective conditions; the transposon excises (RecA independent), giving rise to tetracycline-sensitive segregants. The sequences that flanked the transposon are spliced together during the excision process.

Tn*916* can be reintroduced into *E. faecalis* via transformation of protoplasts (19, 40) by appropriate chimeric plasmids (41). A high-frequency, zygotically induced transposition gives rise to insertions into the recipient chromosome; the plasmid from which the element transposed is generally lost in the process. Genetic studies have shown (35) that when Tn*5* is inserted near the left end of Tn*916*, the tendency to excise under nonselective conditions is eliminated; these derivatives cannot be reintroduced into *E. faecalis*, presumably because they cannot undergo zygotic induction. Since the mutated excision function could be complemented in *trans*, it is conceivable that the related determinant encodes a specific excisase that is integrally related to transposition.

It is clear that the ends of Tn*916* must play a key role in the mechanism of transposition. To gain insight into the nature of this role, we have conducted sequence analyses, the results of which are presented here. We also report analyses of target sequences both before insertion in *E. faecalis* and after excision in an *E. coli* background. The data suggest a possible mechanism for transposition.

(A preliminary account of some of these data was presented at the Second International Conference on Streptococcal Genetics held in May 1986 [23].)

## MATERIALS AND METHODS

**Strains, plasmids, media, and reagents.** Bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains used for plasmid preparation were grown in LB medium (13). When antibiotics were used, they were incorporated in the medium at the following concentrations unless otherwise indicated: ampicillin, 25 μg/ml; tetracycline, 4 μg/ml; and chloramphenicol, 20 μg/ml (all from Sigma Chemical Co.). *E. coli* JM103, used as a host for M13 bacteriophage derivatives, was grown on 2× YT broth (30) or B agar (30) supplemented with isopropyl-β-D-thiogalactopyranoside (156 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (125 μg/ml) (both from Sigma) to detect white plaques resulting from insertional inactivation of the β-galactosidase gene. M13mp18 and mp19 replicative-form DNA was obtained from Bethesda Research Laboratories (BRL) and New England Biolabs, Inc. Klenow fragment of DNA polymerase I, restriction endonucleases, and T4 DNA ligase were from BRL. Avian myeloblastosis virus reverse transcriptase was obtained from Boehringer Mannheim. Deoxynucleoside triphosphates and dideoxynucleoside triphosphates were purchased from Pharmacia, Inc., and radioactive chemicals were from Amersham Corp. Universal sequencing primer (17-mer, catalog no. 1211) was obtained from New England Biolabs. Various specific primers were synthesized by Systec, Inc., Minneapolis, Minn. (Table 2).

**Cloning and sequencing strategies.** DNA fragments for cloning were prepared by restriction enzyme digestion, separation by electrophoresis in a horizontal agarose gel (0.7% in TBE buffer [29]), and recovery by the technique of

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TABLE 1. Strains and plasmids

| Strain or plasmid | Genotype or relevant properties | Plasmid content | Reference, source, or comment |
|---|---|---|---|
| *Enterococcus faecalis* | | | |
| DS16C2 | *tet* (Tn*916*) | pAD1 | 16 |
| FA2-2 | *rif fus* | None | 10 |
| *Escherichia coli* | | | |
| JM103 | Δ(*lac pro*) *thi strA supE endA sbcB hsdR* F′ *traD36 proAB lacI Z*Δ*M15* | F′ | 29 |
| DH1 | F⁻ *recA1 endA1 gyrA96 thi-1 hsdR17 supE44* | None | 29 |
| DH1 FRB | As for DH1 | pBR322::B | This study |
| CG120 | As for DH1 | pAM120 | 18 |
| CG120LT | As for DH1 | pAM120LT | 18 |
| CG160 | As for DH1 | pAM160 | This study |
| CG160LT | As for DH1 | pAM160LT | This study |
| DB11 | *met thi gal hsdR nal rif* | None | J. Davies (28) |
| DB11BS4 | As for DB11 | pACYC184::BS4 | 26 |
| DB11-117 | As for DB11 | pAM117 | 18 |
| Plasmids | | | |
| pAD1 | *hly-bac*, encodes sex pheromone response | | 10 |
| pAM211 | *tet* (Tn*916*) | | pAD1::Tn*916*, insertion in *Eco*RI fragment F (10) |
| pAM1600 | *tet* (Tn*916*) | | pAD1::Tn*916*, insertion in *Eco*RI fragment H |
| pAM717 | *hly-bac* | | Deletion of almost half of pAD1 (21) |
| pGL101 | *bla* | | Derivative of pBR322 (25) |
| pAM120 | *bla tet* (Tn*916*) | | pGL101 carrying *Eco*RI F′ (F::Tn*916*) fragment of pAM211 (18) |
| pAM120LT | *bla* | | pGL101 carrying regenerated *Eco*RI fragment F of pAD1 (18) |
| pAM160 | *bla tet* (Tn*916*) | | pGL101 carrying *Eco*RI H′ (H::Tn*916*) fragment of pAM1600 (this study) |
| pAM160LT | *bla* | | pGL101 carrying regenerated *Eco*RI fragment H of pAD1 (this study) |
| pBR322 | *bla tet* | | 29 |
| pBR322::B | *bla tet* | | *Hin*dIII-*Eco*RI B fragment of pAM120 (left side of Tn*916*) cloned in pBR322 (this study) |
| pACYC184 | *cat tet* | | 29 |
| pACYC184::BS4 | *cat* | | *Bam*HI-*Sal*I fragment of pAD1 (contains *Eco*RI H fragment) cloned in pACYC184 (26) |
| pAM117 | *tet* | | pACYC184 carrying *Eco*RI fragment F of pAD1 (18) |

TABLE 2. Synthetic primers for sequencing (left to right, 5′ to 3′)

| Sequence | Designation | Comments |
|---|---|---|
| TGAGTGGTTTTGACC | Tn-R-O | Anneals in Tn*916*, reads outward from right end |
| TTTGATTCTTGATTT | TnRO-2 | Anneals in Tn*916*, reads outward from right end |
| GTGAAGTATCTTCCTAC | TnLO-2 | Anneals in Tn*916*, reads outward from left end |
| CATGACGCTGAACTATT | TnLO-3 | Anneals in Tn*916*, reads outward from left end |
| GGTCAAAACCACTCA | 56T7 | Anneals in Tn*916*, reads inward from right end |
| TGTGCTTTCGAGTAT | Tn-L-I | Anneals in Tn*916*, reads inward from left end |
| GGCAAAAGGAAACAG | HTH-R | Anneals in *Eco*RI fragment H of pAD1, reads toward right end of Tn*916* or through target site |
| GCAATAGGAATTAAGAG | HTE-L | Anneals in *Eco*RI fragment H of pAD1, reads toward left end of Tn*916* or through target site |
| TGAATATACCAGGCA | FTE-R | Anneals in *Eco*RI fragment F of pAD1, reads toward right end of Tn*916* or through target site |
| TATACTGGCTTAGAG | FTB-L | Anneals in *Eco*RI fragment F of pAD1, reads toward left end of Tn*916* or through target site |

Benson (2). Ligation, preparation of *E. coli* JM103 competent cells, and transfection with M13 derivatives was as recommended by BRL.

In the construction of pAM160, *Eco*RI fragment H of pAD1 containing Tn*916* was cloned in pGL101 (from pAM1600) by a previously described method (18). Subsequent cloning procedures were as follows. *Hin*cII junction fragments from pAM160 were digested with *Alu*I and shotgun-cloned into M13mp18 at the *Sma*I site. Clones containing the junction were identified by Southern hybridization (37) of plaque lifts (3) by using a $^{32}$P-labeled probe consisting of pAM717 (a deletion derivative of pAD1 which retains *Eco*RI fragments F and H). To identify clones containing the left end of Tn*916*, hybridization was also carried out with a probe specific for the left end (*Eco*RI-*Hin*dIII fragment B of pAM120). Probes were labeled with [α-$^{32}$P]dATP (800 Ci/mmol) with a nick translation kit (BRL). Autoradiography was carried out at −70°C with Kodak X-Omat AR film and a Du Pont Cronex Lightning-Plus intensifying screen. Sequencing was performed with a universal M13 primer, and the Tn*916* right-hand junction was identified by comparison with the insertion target in *Eco*RI fragment H, which was cloned according to the following scheme. A ~500-base-pair (bp) *Eco*RI-*Hin*cII restriction fragment of pAM160LT (representing the target after Tn*916* excision) or pACYC184::BS4 (containing the H fragment before insertion) was cloned into M13mp18 or M13mp19. This was done either by specific *Eco*RI-*Hin*cII cloning or by insertion of the entire *Eco*RI fragment H and subsequent deletion of a *Hin*cII fragment, placing the *Hin*cII site of H close to the universal priming site. Sequencing was performed with a universal primer.

After the Tn*916* right junction in H and the H insertion site were identified, specific primers were synthesized which would anneal close to the insertion site and read through it in both directions. The same primers would allow readthrough of the Tn*916* junction regions in clones containing the ends of the transposon. The left junction of Tn*916* was sequenced in this manner by using M13 derivatives containing inserts too large to allow sequencing of the desired region by the use of universal primer. The specific primers were also used to derive sequence data on the insertion site (after excision) directly from plasmid pAM160LT.

The outward-reading Tn*916*-specific primers were used to read through the transposon junctions for the Tn*916* insertions in *Eco*RI fragment F of pAD1. For the right side, a *Bam*HI-*Hin*cII restriction fragment (~1 kb) containing the junction from pAM120 was cloned in opposite orientations into M13mp18 and M13mp19. To sequence the left junction, plasmid template pBR322::B was used. By reading outward from Tn*916*, sequence information about the bordering F segments was obtained. Specific primers were made to read back through the junction in the opposite direction or to read through the insertion site in appropriate clones. Clones containing the insertion site in F were generated as follows. A 1.8-kb *Bam*HI-*Eco*RI restriction fragment from pAM117 (pACYC184 carrying the F fragment of pAD1) and the comparable fragment after excision of Tn*916* (from pAM120LT) were each cloned into both M13mp18 and M13mp19. In some cases, sequencing was performed directly on a plasmid template of pAM120LT.

**Sequencing and related protocols.** Dideoxy sequencing with M13 clones was performed by published methods (4, 33, 34; BRL M13 cloning and sequencing manual). Reactions were carried out at 30°C with [α-$^{35}$S]dATP (>600 Ci/mmol) as the label. Separation of DNA fragments was done by electrophoresis through vertical 6% polyacrylamide gels (40 by 32 by 0.05 cm) containing 42% urea, after which bands were visualized by autoradiography at ambient temperature on Fuji RX film.

Direct sequencing from double-stranded plasmid templates employed the following methods: (i) an alkaline denaturation method described by Chen and Seeburg (6); (ii) a modification of the M13 method, in which template DNA is linearized and annealed to primer by boiling followed by rapid cooling on ice (New England Biolabs protocol); (iii) the reverse transcriptase method of Zagursky et al. (42) as modified by Bartlett et al. (1); and (iv) the Sequenase system (United States Biochemical Corp.). The Sequenase kit was adapted for use with plasmid templates by annealing primer and template molecules by an alkaline denaturation method, essentially the same as that of Zagursky et al. (42).

Plasmid templates of pGL101 and pBR322 vector derivatives were prepared by the following method. LB broth (500 ml) plus an appropriate antibiotic(s) was inoculated with 5 ml of an overnight culture and incubated with vigorous shaking (200 rpm) at 37°C until late log phase. Chloramphenicol (200 μg/ml) was added, and incubation as above was continued overnight. The culture was centrifuged at 4,400 × *g* for 10 min at 4°C, and the resulting cell pellet was suspended in 30 ml of TE (50 mM Tris hydrochloride, 50 mM EDTA, pH 7.5). Lysozyme (6 mg; Sigma) was added, and the suspension was placed on ice for 1 h. Triton X-100 (Sigma; 0.6 ml of a 20% aqueous solution) was added, followed by incubation at 37°C for 15 min with slow shaking. After a clearing spin (44,000 × *g* 1 h, 4°C), the supernatant was drawn off and the plasmid DNA was recovered after two cycles of isopycnic CsCl-ethidium bromide gradient centrifugation. Preparation of other plasmids was done by a crude lysate method involving treatment with lysozyme, pronase (Sigma), and Sarkosyl NL30 (ICN Pharmaceuticals, Inc.), followed by CsCl-ethidium bromide gradient centrifugation as above. In some cases, plasmid DNA was prepared by the rapid method of Zagursky et al. (42).

Analysis of sequence information was performed with the DNA Inspector II+ program (Textco) and a Macintosh Plus computer. All data for the insertion sites (both before insertion and after excision of Tn*916*) were obtained from sequences read in both directions. Sequence data for the transposon termini were generated in both directions, except for the bases designated 1 through 57 on the right end (see Fig. 1B).

**Generation of pAD1::Tn*916* insertions.** Hyperhemolytic derivatives resulting from insertions of Tn*916* into pAD1 were generated as described previously (16, 17). Briefly, *E. faecalis* DS16C2 was used as a donor in overnight filter matings with recipient strain FA2-2. Transconjugants were selected on blood-agar plates containing tetracycline (12.5 μg/ml) in addition to antibiotics specific for the recipient. Colonies exhibiting a zone of hemolysis with a diameter three to four times the normal size were analyzed further to determine the location and orientation of Tn*916* by restriction enzyme analysis.

## RESULTS

Two different *E. coli* chimeric plasmids were used in the DNA sequencing analyses. Each corresponded to the vector pGL101 into which a different *Eco*RI fragment containing Tn*916* had been cloned from pAD1::Tn*916* derivatives (Table 1; Tn*916* has no internal *Eco*RI sites). The two pAD1 *Eco*RI fragments F and H contained different parts of the hemolysin-bacteriocin determinant(s) of pAD1 (10); the in-

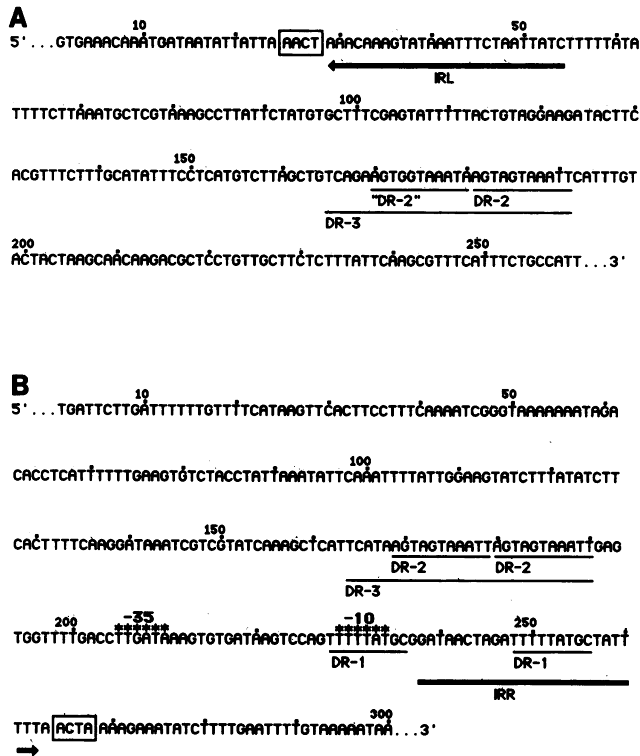


FIG. 1. Nucleotide sequence of left (A) and right (B) ends of Tn*916*. The sequences relate to the insertion present in the pAM160 chimera. Junction regions are boxed. Inverted repeat (imperfect) sequences (IRL and IRR) and the direct repeat sequences DR-1, DR-2 (and "DR-2"), and DR-3 are underlined. Potential promoter hexamers (−10 and −35 sequences) within the right end are indicated by asterisks.

sertions were originally derived by screening for elimination of hemolysin expression (16, 17). The first analyses were done with pAM160, a chimera containing the *Eco*RI H::Tn*916* fragment (Fig. 1). The termini were initially localized by comparisons with the sequence of original target DNA; however, comparisons with the other insert (relating to pAM120, Fig. 2) revealed the junction regions by the presence of terminal transposon sequences constant in both insertions. In the case of pAM160, about 230 nucleotides of the left end of the transposon and about 260 nucleotides of the right end (Fig. 1) were sequenced. The left end had an overall G+C content of 32% (18% in the first 50 bp), whereas the right end was 28%. (The overall G+C content of *E. faecalis* chomosomal DNA is 38%.) The boxed-in areas (Fig. 1 and 2) indicate the transposon-plasmid junctions. In each case the surrounding regions contained directly repeated homologous sequences; however, the length of this homology was 7 and 6 nucleotides for pAM160 and pAM120, respectively. In the case of pAM120, two nonadjacent C's on the left side interrupted an otherwise homologous 19-bp region (Fig. 2).

Analyses of the target site both before insertion and after excision (in *E. coli*) indicated that the sequence after excision could differ from the sequence prior to insertion. For example, in the case of pAM160, in which the transposon was inserted into the pAD1 *Eco*RI fragment H, the fragment after excision could differ from its original sequence by 3 bp (Fig. 3A). In this case the excision product had a chance of containing either CTA or ACT. This was evidenced by the fact that two different M13 subclones yielded the two different sequences. Also, when a sequencing analysis was performed directly on plasmid DNA corresponding to a tetracycline-sensitive segregant, a mixture of the two sequences was observed. The presence of two forms of the sequence may reflect the fact that the chimera generally exists as a multimeric plasmid (data not shown) and that excision occurred two different ways. Interestingly, the CTA and ACT sequences were found also in the right and left junction regions, respectively, of Tn*916* in the pAM160 chimera (Fig. 1). It would therefore appear that one of the two sequences (CTA) was brought in with the transposon or was generated as a result of the insertion process.

A similar result was observed in the pAM120 chimera. As shown in Fig. 3B, there was an ATA present in the original target which was replaced by CAT in the plasmid excision product. Analysis directly on plasmid DNA from a tetracycline-sensitive segregant showed only the CAT form. As in pAM160, the two sequences could be found in the junction regions at opposite ends of the transposon.

**Characteristics of the sequence.** The two ends of the transposon contained imperfect inverted repeats with identity at 20 of 26 nucleotides (Fig. 1). These are referred to as IRL (inverted repeat left) and IRR (inverted repeat right). The right end of Tn*916* also contained two sets of short direct repeats, designated DR-1 and DR-2 (Fig. 1). The two DR-1 sequences were 9 bp long and separated by 11 bp. The DR-2 sequences were 11 nucleotides long and were contiguous. Another set of contiguous DR-2 repeats appeared near the left end of the transposon; however, one segment (indicated as "DR-2") differed by 2 bp. Within each end of the transposon was a direct repeat, designated DR-3, which was 27 nucleotides long with differences at only three positions. These two repeats contained the DR-2 sequences within them. The left DR-3 sequence was about 135 nucleotides from the terminus; the right DR-3 was about 75 nucleotides from the terminus.

A potential outward-reading promoter site was present in the right end of the transposon adjacent to IRR. As shown in Fig. 1, the −10 hexamer sequence occurred within DR-1 and was separated from the −35 hexamer by 18 nucleotides.

**On the relationship between Tn*916* and hyperhemolytic expression of pAD1.** The presence of a potential promoter site on one end of Tn*916* suggested that it might be involved

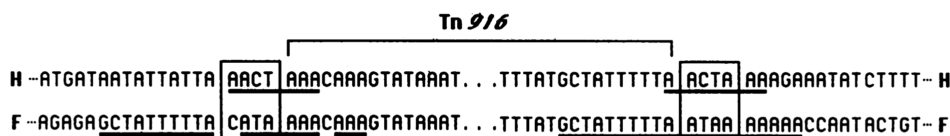


FIG. 2. Junction regions of two Tn*916* insertions. The sequences relate to the insertions of pAM160 and pAM120. Boxed segments contain the junction regions. Underlined regions represent directly repeated homology at opposite ends. F and H indicate extensions into sequences originally corresponding to the *Eco*RI F and H fragments, respectively, of pAD1.

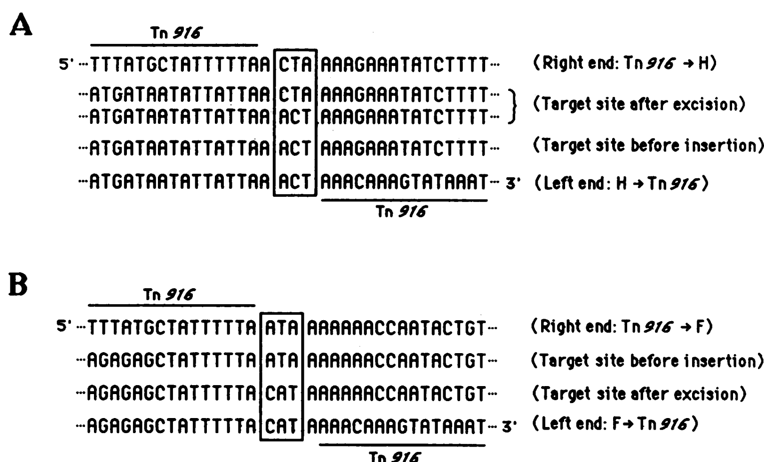


FIG. 3. Sequence of target sites both before insertion (in *E. faecalis*) and after excision (in *E. coli*) and comparison with the related insertion junction regions. The sequences relate to those of pAM160 (A) and pAM120 (B). In the case of pAM160 after excision, two different sequences were detected in similar amounts. In the case of pAM120, only one version was detected after excision.

in the transposon's ability to give rise to hyperexpression of hemolysin upon insertion into pAD1. If this were indeed the case, it would imply that all inserts exhibiting this phenomenon would be oriented so that the putative promoter would read toward the hemolysin determinant. To see whether this was the case, a number of pAD1::Tn*916* derivatives with hyperhemolytic expression were generated so that the orientation of the insertions could be determined. For some of these derivatives, hyperhemolytic expression was facilitated by growing the cells in the presence of tetracycline, whereas others did not require the presence of the drug. (The tetracycline effect is believed to be indirect and may relate to a stress phenomenon; see reference 9.) Mapping of 18 independently obtained inserts from strains of the hyperhemolytic phenotype revealed that all occurred in the same general location. Within the limits of detection, it was not possible to distinguish between the locations of the inserts; all mapped at about 0.95 kb from the *Eco*RI site separating the H and D fragments at about 35.3 kb on the pAD1 map (14). Most important, it was found that all the insertions were indeed oriented so that the right end of the transposon was proximal to the hemolysin determinant. It is possible, if not likely, that many of these inserts were identical, reflecting site-specific insertion; however, since at least two different phenotypes were involved, as determined by the effect of tetracycline, insertions were probably occurring at more than one site.

## DISCUSSION

It was proposed previously that Tn*916* moves via an excision-insertion mechanism involving a circular intermediate (17, 18). Support for excision comes with the behavior of the transposon when residing as a passenger on a conjugative plasmid in *E. faecalis* (17). Conjugal transfer of such a plasmid results in a zygotic induction, leading to excision of the element from the plasmid and its subsequent loss (segregation) or insertion into the chromosome. It appeared that the excision could be precise, since in a pAD1 derivative it restored normal expression of the hemolysin gene.

It has also been proposed (18) that the high level of excision of Tn*916* from plasmid chimeras that occurs in *E. coli* may represent an aberrant transposition involving only the first step (excision). The data presented here show that in the case of one of the two insertions, excision in *E. coli* regenerated the original target sequence some of the time. Alternatively, there was a 3-bp change at the excision site. For the other insertion, a 3-bp change in the excision product was the only form detected. It would appear significant that in both cases when the transposon was present, one junction site contained the sequence corresponding to the target before excision, while the other junction site contained that corresponding to after excision.

One can envision a model such as that shown in Fig. 4, where Tn*916* is seen as a hypothetical circular intermediate with a tetrameric "core" sequence which may serve as a "spacer" between the two ends of the transposon. (A tetrameric rather than a trimeric core is postulated in order to reconcile the two different insertions, whose specific trimers overlap by 2 bp.) When the core sequence (indicated as XXXX in Fig. 4) is lined up with the two target sequences involved in this study, a significant degree of homology in adjacent nucleotides can be observed. A reciprocal recombination on either side of the opposing core sequences would give rise to insertions with the two tetramers located at opposite ends, as was observed from the sequence data. A reversal of the process would lead to an excision that would

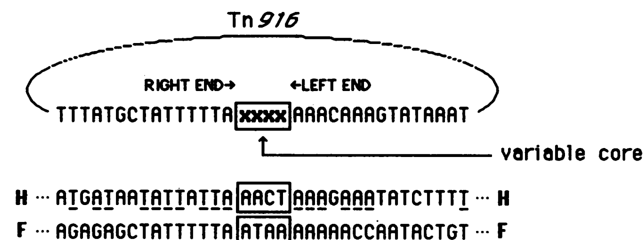


FIG. 4. Possible model for transposition of Tn*916*. The transposon is shown as a circular intermediate with a tetrameric region referred to as a variable core region. The latter region may line up with a corresponding, but not necessarily identical, tetramer in the target. If the two tetramers are different, recombination on either side would lead to two different "tetramers" at opposite ends of the insertion—one of which was brought in by the transposon. Excision would correspond to a reversal; however, different tetramers may be left behind, depending on which side of the opposing cores recombination takes place. The individual bases underlined indicate homologies with corresponding bases in the ends of the transposon.

leave one of the tetramers behind, depending on the position of the recombination event. The degree of homology occurring on either side of the core tetramer may influence where the recombination actually takes place. Movement of Tn*916* from one site to another could therefore involve exchange of a tetrameric sequence originally present in the target replicon with a sequence that had been brought in by the transposon. It is also possible that some insertions would involve a target core sequence identical to that of the tetramer brought in by the transposon; such inserts would have identical junction sequences.

The terminal sequences of the pneumococcal transposon Tn*1545* have recently been reported by Caillaud and Courvalin (5) and are essentially identical to those of Tn*916*. The left end of Tn*916* corresponds to the "right" end of Tn*1545*, with identity at 184 of the 186 bases reported; the other ends are identical for the 108 bases reported for Tn*1545*. One of the two target sequences determined here for Tn*916* (in *Eco*RI fragment F of pAD1) contains a "consensus" target deduced for Tn*1545* (5), and the other target bears some similarity. It is evident that, like the transposon termini, target regions are very AT rich.

Insertions of Tn*1545* also exhibited terminal variable base pairs involving four nucleotides. In the case of Tn*1545*, however, excision appeared to be "clean," leaving the target sequence essentially unchanged from that prior to insertion. The absence of exact duplications of target sequences by these transposons distinguishes them from many other transposons (20, 24). The staphylococcal transposon Tn*554* (nonconjugative) is similar in this regard (31, 32).

*E. faecalis* derivatives with Tn*916* located at different sites in the chromosome are known to differ significantly in their donor potential and can give rise to transconjugants at frequencies of $10^{-9}$ to $10^{-5}$ per donor, depending on the donor (16, 17). There is a direct correlation between the ability to transpose intercellularly and intracellularly (i.e., to a resident plasmid) (17); we believe that this is due to a common excision step, the frequency of which is characteristic of the specific insert. It is apparent (e.g., Fig. 4) that the difference in excision potential may be influenced by the degree of homology between the ends of the transposon and sequences outside the insertion. Other factors, such as read-in from outside promoters (e.g., into the hypothetical excisase on the left end of the transposon), could also influence the frequency of transposition.

The occurrence of a potential promoter site(s) on the right end of Tn*916* may be significant in light of the fact that insertions adjacent to the hemolysin gene(s) of pAD1 frequently exhibit hyperexpression of hemolysin. (On blood plates the zone of hemolysis is usually three to four times the diameter of that with the wild type.) The hyperexpression of hemolysin may be due to read-in from such a promoter, an interpretation supported but not proved by the finding that a number of such derivatives examined all had the right end of the transposon proximal to the hemolysin determinant. It is interesting that practically all conjugative transposons examined have been able to generate the hyperhemolytic phenotype as a result of certain insertions into pAD1 (9), a phenomenon never observed in hundreds of Tn*917* (a member of the Tn*3* family) insertions (14, 21).

Since the terminal nucleotide sequences of the pneumococcal conjugative transposon Tn*1545* are essentially identical to those reported here for Tn*916*, it appears likely that many, if not most, conjugative transposons have similar sequences. This not only suggests that there is a common overall mechanism of transposition but points to the likely basis for why all of them are able to generate the hyperhemolytic phenotype.

## ACKNOWLEDGMENTS

We thank P. Schultz and K. Sippell for valuable technical assistance.

This work was supported by Public Health Service grants AI-10318 and DE-02731 from the National Institutes of Health. C.G.-B. was the recipient of a Junior Faculty Research Award from the American Cancer Society while at the University of Michigan.

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