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Length of the Coupling Sequence of Tn*916*

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The coupling sequences of conjugative transposons are short variable sequences derived from the DNA flanking the transposon insertion site. We show here that for Tn*916* the left coupling sequence is 6 bases long. The right-hand end of the transposon can excise with either four or five T's, but integration occurs to restore the five T's at the transposon's right end.

Conjugative transposons are closely related mobile DNA elements found in both gram-positive and gram-negative bacteria. Gram-positive bacteria containing these elements act as donors in a conjugation event in which the transposon is transferred to the recipient organism. Transfer of these elements is promiscuous, since it is as efficient when the mating partners are bacteria of different genera as when they are of the same genus. Most conjugative transposons carry *tetM* or a similar tetracycline resistance gene. The smallest such element, Tn*916*, is 16.4 kb, and the larger elements, like Tn*1545*, usually carry additional resistance genes. Often, conjugative transposons are found in clinical isolates of pathogenic bacteria, so their ability to spread antibiotic resistance among members of different genera is a serious medical problem. Recent reviews of conjugative transposons include those by Clewell and Flannagan (5) and Scott (12, 13).

The mechanism by which conjugative transposons like Tn*916* move differs from that of the other classes of transposons. One of the first differences recognized is that integration of conjugative transposons does not result in duplication of the target sequence (6). The mechanism of transposition of these elements is similar to that of lambda and related prophages in that it utilizes a protein of the lambda integrase family to excise the element and produce a covalently closed circular intermediate molecule (9, 15). Like lambda, these transposons appear to be excised from the host DNA by staggered endonucleolytic cleavages. However, in contrast to lambda, the "overhangs" resulting from these cleavages are composed of bases that flanked the transposon in the host DNA. These are called coupling sequences, and their composition is not specific. Because the coupling sequences on the two strands are not complementary, the circular transposon intermediate molecule contains mismatches at the joint (3). This represents an important difference from lambda because such mismatches prevent the branch migration required for recombination. Therefore, the basic enzymatic mechanism of Tn*916* transposition must have important differences from that of lambda.

A definition of the precise transposon ends, and therefore of the exact length of the coupling sequence, has remained elusive. The ends have been defined in relation to an imprecise inverted repeat, in which 20 of 26 bases are identical (6). In Clewell's designation of Tn*916*, this places a run of T's at the right end of the transposon. However, in each of the laboratories that work with Tn*1545* or Tn*916*, the number of T's at this end has been found to vary (2, 3, 6, 10).

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The circular transposon molecule with mismatched coupling sequences integrates into a target site by a process that appears to be the reciprocal of excision. Unlike lambda, the insertion target for Tn*916* is not specific. However, transposon target sites are not chosen randomly either, and the same preferred targets are used regardless of the specific bases in the coupling sequence of the donor transposon. Although there are additional (as yet unknown) characteristics that make a target a "hot spot," targets seem to consist of a stretch of A's separated from a T-rich region by a group of 5, 6, or 7 bases (10, 14, 16). The coupling sequence comes from these bases. Because of the T-rich nature of the target, it has been difficult to distinguish how many T's belong to the transposon, how many to the coupling sequence, and how many to the target.

Since the transposon ends are defined arbitrarily, the length of the coupling sequence has also been interpreted differently to be 4 (2, 6), 5 (3), 5 or 6 (8), or 6 or 7 (10) bases. This ambiguity results largely from the AT-rich nature of the target sequences chosen by the transposon for insertion and the variable number of T's at the right end of the transposon. Recently, using a minitransposon model system and a synthetic target in *Escherichia coli*, Trieu-Cuot et al. (16) demonstrated that the coupling sequence is 6 bases (Fig. 3; I6$^-$ in reference 16). In the experiments presented below, using natural conjugative transposition in a gram-positive system, we have also found that the coupling sequence is 6 bases.

By analyzing the sequences surrounding Tn*916* in the donor and in transconjugants, we recently deduced that only one specific strand of the transposon is transferred during conjugation (14). Recircularization of this strand and complementary strand synthesis after transfer generate a circular intermediate (without mismatches) which then integrates into the new host's DNA. One of the key observations in this work was that following conjugational transposition of Tn*916*, only the coupling sequence that was adjacent to the left side of the transposon (as defined by Clewell et al. [6]) in the conjugational donor was found in the transconjugants. Therefore, when the sequence surrounding Tn*916* in the donor is known, the coupling sequence that will be transferred to a recipient in conjugation can be identified. With this knowledge, we have designed two experiments to differentiate between a 5-base coupling sequence and a 6-base coupling sequence and between a 6-base coupling sequence and a 7-base coupling sequence, respectively. Furthermore, once the length of the coupling sequence was known, the precise end of the transposon could be determined.

Transconjugants were generated by mating either of two *Enterococcus faecalis* donors (14) with the *Bacillus subtilis* W168 Fus2 strain on filters overnight (11). To ensure independent generation of the transconjugants studied, only one was

FIG. 1. Sequence of donors and transconjugants showing the expected and observed coupling sequences from two matings. Transposon bases (italics), the coupling sequence that was present in the donor (underlined), and the sequence of the target before insertion (boldface print) are indicated. Distinctions between 5- and 6-base coupling sequences (donor DMM105) (A) and between 6- and 7-base coupling sequences (donor DMM108) (B) are indicated by double underlines.

picked from each filter. It is highly likely that these transconjugants are the products of only one round of excision and integration, because the frequency of spontaneous excision of Tn*916* is low (between 10^{-5} and 10^{-8}; data not shown). Even if additional excision and insertion events of the same coupling sequence had occurred, they would not affect the conclusions about the length of a coupling sequence. Analysis by Southern blot showed that each transconjugant contained only one copy of Tn*916* in its genome (data not shown). DNA was obtained from each transconjugant, and the ends of the transposon and the flanking DNA were sequenced with the double-stranded DNA cycle sequencing system (Gibco) by using primers specific for each end of Tn*916*.

The first donor chosen (strain DMM105) had the sequence CACAGG left of the transposon. Thus, whether the coupling sequence was 5 or 6 bases, the last base of the coupling sequence was not a T and it could therefore be distinguished from the right end of Tn*916*. Fig. 1A shows the sequence flanking the transposon in the donor, the coupling sequence expected if it were 5 bases and if it were 6 bases, and the sequences observed in two transconjugants. In transconjugant 1, the transposon had been inserted into the region between *flhA* and *flhB* (4), whose sequence was found in the GenBank data base. Therefore, the target sequence shown in Fig. 1A for transconjugant 1 was independently known. The target sequence for transconjugant 2 was not identical to any sequence in the data base. However, it could be deduced because an insertion into the same target was found in a mating with a different donor strain (Fig. 1B). In both transconjugants 1 and 2, the sequence surrounding the inserted transposon is consistent with a coupling sequence of 6, but not of 5, bases. Four other independently derived transconjugants from the same cross also had the coupling sequence CACAGG; the sequence ACAGG was never seen.

Matings with the donor strain DMM105 will not distinguish between 6- and 7-base coupling sequences because the next base adjacent to the coupling sequence in the donor is a T. It

TABLE 1. Cases where the transposon has excised with four and five T's

No. of T's	Sequence[a]	Origin	Reference
4	...*TATTTT* **TATTATAAAAA**	DMM102	14
	...*TATTTT* *TAAAAT***AAAAA**	DMM103	14
	...*TATTTT* *TAAAAT***AAAAA**	DMM105	14
	...*TATTTT* *TCTAAG***AAAAA**	DMM106	14
	...*TATTTT* *TAAAAT***AAAAA**	DMM107	14
	...*TATTTT* *TCTAAG***AAAAA**	DMM108	14
	...*TATTTT* **TAAATCGAAAA**	CKR51	This work
	...*TATTTT* **TCTGTATAAAA**	CKR61	This work
	...*TATTTT* *TCTAAG***AAAAA**	CKR201	This work
	...*TATTTT* **TATAATAAAAA**	CKR211	This work
	...*TATTTT* *TTTGAT***AAAGA**	CKR221	This work
	...*TATTTT* *TAGCTA***AAAAA**	BUG5	7
	...*TATTTT* *TAGCTA***AAAAT**	BUG13	7
	...*TATTTT* **TAATAAAAAAA**	pAM120	6
	...*TATTTT* *TAACTA***AAAGA**	pAM160	6
	...*TATTTT* **TATCTATAAAA**	pIP806	2
5	...*TATTTTT* **TTTATAATAA**	DMM104	14
	...*TATTTTT* **GCTTATTAAA**	FB30	14
	...*TATTTTT* **CCTGTGAAAA**	FB31	14
	...*TATTTTT* *CACAGG***AAAA**	CKR71	This work
	...*TATTTTT* *CACAGG***AAAA**	CKR81	This work
	...*TATTTTT* *TAACTA***AAAA**	pAM70-11	8
	...*TATTTTT* **TATCTATAAA**	pAM70-17	8
	...*TATTTTT* **ATCTATAAAA**	pAM210	8
	...*TATTTTT* *TAAAAT***AATC**	pAT303 (I3)	10
	...*TATTTTT* *TAAAAT***AATA**	pAT304 (I4)	10

[a] The sequence from the right end of the transposon is shown in italics, the coupling sequence from the donor is underlined, and the target sequence is in boldface print.

is not clear how many T's are at the right end of the transposon, and all targets investigated so far are T-rich, so the origin of a T in this region cannot be unambiguously determined. Therefore, a second donor (strain DMM108) with a flanking sequence that would distinguish between coupling sequences of 6 (TTTGAT) and 7 (ATTTGAT) bases was chosen. The A in the seventh position of the coupling sequence makes this possible.

Fig. 1B shows the sequence of the donor DMM108, the expected 6- and 7-base coupling sequence, and the sequence in one transconjugant. The A that would be introduced into the target if the coupling sequence were 7 bases is clearly not present in the transconjugant. Thus, it can be concluded that the coupling sequence was 6 and not 7 bases. The same 6-base coupling sequence was observed in a second independent transconjugant in which the transposon had integrated at a different target site (data not shown). Although no independent determination of the target sequence exists, we can determine the coupling sequence because it is next to the right side of the transposon and because the 7th base, which is an A, is not present between the T's which make up the end of the transposon and the coupling sequence.

From these studies, we conclude that the left coupling sequence in Tn*916* conjugative transposition is 6 bases long. This presumably means that the enzyme responsible for transposon excision nicks the two DNA strands at sites which are 6 bases apart, resulting in 6-base overhangs. Since we do not see the right coupling sequence, it is still possible that it is 6 or 7 bases. However, as there has never been any indication of pairing of different-size coupling sequences (3), we assume the right and left coupling sequences are the same length, that is, 6 bases. Furthermore, a coupling sequence of 6 bases is consistent with all of the published sequences of transposon ends and target sites (compiled and reported in reference 14).

The location of the right-hand end of the transposon can be deduced once the number of bases in the coupling sequence and the sequence of the target site are known. Fig. 1A shows that there are sometimes four and sometimes five T's on the right-hand end of Tn*916*. A similar variation in the number of T's was previously attributed to imprecision by the excision enzyme when counting T's at the end of the transposon (3). Imprecise excision does not alter the length of the coupling sequence but results in an extra base remaining in the excisant after the transposon leaves (3). It also indicates that the end of the inverted repeat on the right of the transposon does not always mark the site for endonucleolytic cleavage and brings into question the role of these repeats.

Further analysis of the target sites after insertion shows that there is always a minimum of five T's at the right end of the transposon. When only four T's have been inherited from the transposon, the fifth T is present in the target or in the coupling sequence. In Table 1, we present a compilation of sequences flanking Tn*916* in which the right transposon end, the adjacent coupling sequence, and the target can be distinguished. In addition to these 26 cases, there are 11 others in which the transposon end cannot be distinguished unambiguously, and all of these also have at least five T's in this location. Thus, no case has ever been reported in which there are fewer than five T's on the right of the transposon. Although the meaning of this observation is not yet apparent, it must be explained in any detailed mechanism proposed for insertion of conjugative transposons. Biochemical characterization of the reaction involved is required to elucidate the role of these T's.

The sequences obtained in this study were compared with published sequences by searching the data bases at the National Center for Biotechnology Information via the BLAST network service (1).

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REFERENCES

1. **Altschul, S., W. Gish, W. Miller, E. Myers, and D. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Caillaud, F., and P. Courvalin.** 1987. Nucleotide sequence of the ends of the conjugative shuttle transposon Tn*1545*. Mol. Gen. Genet. **209**:110–115.
3. **Caparon, M. G., and J. R. Scott.** 1989. Excision and insertion of the conjugative transposon Tn*916* involves a novel recombination mechanism. Cell **59**:1027–1034.
4. **Carpenter, P., and G. Ordal.** 1993. *Bacillus subtilis* FlhA: a flagellar protein related to a new family of signal-transducing receptors. Mol. Microbiol. **7**:735–743.
5. **Clewell, D. B., and S. E. Flannagan.** 1993. The conjugative transposons of Gram-positive bacteria, p. 369–393. *In* D. B. Clewell (ed.), Bacterial conjugation. Plenum Press, New York.
6. **Clewell, D. B., S. E. Flannagan, Y. Ike, J. M. Jones, and C. Gawron-Burke.** 1988. Sequence analysis of termini of conjugative transposon Tn*916*. J. Bacteriol. **170**:3046–3052.
7. **Gaillard, J.-L., P. Berche, C. Frehel, E. Gouin, and P. Cossart.** 1991. Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from Gram-positive cocci. Cell **65**:1127–1141.
8. **Ike, Y., S. E. Flannagan, and D. B. Clewell.** 1992. Hyperhemolytic phenomena associated with insertions of Tn*916* into the hemolysin determinant of *Enterococcus faecalis* plasmid pAD1. J. Bacteriol. **174**:1801–1809.
9. **Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, and P. Courvalin.** 1989. Molecular characterization of two proteins involved in the excision of the conjugative transposon Tn*1545*: homologies with other site-specific recombinases. EMBO J. **8**:2425–2433.
10. **Poyart-Salmeron, C., P. Trieu-Cuot, C. Carlier, and P. Courvalin.** 1990. The integration-excision system of the conjugative transposon Tn*1545* is structurally and functionally related to those of lambdoid phages. Mol. Microbiol. **4**:1513–1521.
11. **Sasaki, Y., N. Taketomo, and T. Sasaki.** 1988. Factors affecting transfer frequency of pAMB1 from *Streptococcus faecalis* to *Lactobacillus plantarum*. J. Bacteriol. **170**:5939–5942.
12. **Scott, J. R.** 1992. Sex and the single circle: conjugative transposition. J. Bacteriol. **174**:6005–6010.
13. **Scott, J. R.** 1993. Conjugative transposons, p. 597–614. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria. American Society for Microbiology, Washington, D.C.
14. **Scott, J. R., F. Bringel, D. Marra, G. Van Alstine, and C. K. Rudy.** 1993. Conjugative transposition of Tn*916*: preferred targets and evidence for conjugative transfer of a single strand, and for a double-stranded circular intermediate. Mol. Microbiol. **11**:1099–1108.
15. **Scott, J. R., P. A. Kirchman, and M. G. Caparon.** 1988. An intermediate in transposition of the conjugative transposon Tn*916*. Proc. Natl. Acad. Sci. USA **85**:4809–4813.
16. **Trieu-Cuot, P., C. Poyart-Salmeron, C. Carlier, and P. Courvalin.** 1993. Sequence requirements for target activity in site-specific recombination mediated by the Int protein of transposon Tn*1545*. Mol. Microbiol. **8**:179–185.