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Effects of Deletions in Transposon Tn7 on Its Frequency of Transposition

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Deletions in transposon Tn7 either abolished transposition or reduced transposition frequency. Except for a deletion in the right-hand terminus, these deletions could be complemented in *trans*. A 2.1-kilobase fragment of Tn7 encodes a diffusible gene product which stimulates transposition above the wild-type frequency. No cointegrate formation was detected.

Transposon Tn7 is a large (14-kilobase [kb]) transposable element which codes for resistance to trimethoprim and streptomycin-spectinomycin (Tp^r Sm^r/Sp^r) (2, 6). Its high frequency of transposition into a variety of plasmids and bacterial chromosomes has made it a useful tool for the generation of insertion mutants (3, 4, 7, 11, 15, 21, 22).

Insertion of Tn7 into the *Escherichia coli* chromosome occurs at a specific site, in only one orientation and at a high frequency (1, 2, 20). The insertion site and the termini of Tn7 have been sequenced (19). There is evidence that there are also preferred sites for the insertion of Tn7 into the chromosomes of other genera (7, 14, 15, 21, 23).

The frequency of transposition of Tn7 into plasmids is lower than that into the *E. coli* chromosome; although there appears to be no specific site for insertion, this is always in one orientation (3).

We report here the effects of several deletions in Tn7, made by using restriction endonucleases, on its transposition into the IncP plasmid pUZ8, which is believed to contain no transposable elements (17). We also report the complementation in *trans* of some of the transposition-deficient deletions by plasmids containing cloned fragments of Tn7.

Deletions in Tn7 (Fig. 1) were made from the plasmid ColE1::Tn7 (13, 18). Their construction was facilitated by the fact that plasmid ColE1 (13) contains no sites for restriction endonucleases *Bam*HI, *Bgl*II or *Hin*dIII, all of which have two or more sites in Tn7. The deletions were made by standard techniques (20). Plasmid DNA was prepared from detergent lysates (9), using ethidium bromide-cesium chloride equilibrium centrifugation. The DNA was cleaved with the appropriate restriction endonucleases (Bethesda Research Laboratories), the fragments were separated by agarose gel electrophoresis, and the required fragments were purified, ligated, and used to transform a restrictionless strain of *E. coli* K-12, strain M259. The structures of these plasmids were confirmed by restriction endonuclease digestion of small-scale plasmid DNA preparations (5) and analysis with agarose gel electrophoresis (20).

Fragments of Tn7 from plasmids ColE1::Tn7 and pCPL6::Tn7 (19) were cloned into the small multicopy plasmid vector pACYC184 (8) (Fig. 1) by standard techniques (20), and the structure of the plasmids was confirmed as described above.

The efficiency of transposition of the deleted transposons into the conjugative plasmid pUZ8 was estimated. *E. coli* K-12 strain JA221 (*recA*), containing the deleted plasmids and pUZ8, was mated with recipient strain J62 (10), and the transposition frequency was expressed as the number of Tp^r transconjugants per pUZ8 transconjugant. Plasmids containing cloned fragments of Tn7 to be tested for complementation of transposition functions were introduced by transformation into *E. coli* strains containing the deleted plasmids and pUZ8, and the transposition frequency was estimated in the same way. In each case, 100 Tp^r transconjugants, or all of them if there were less than 100, were tested for colicinogeny (10). This was to test for any mobilization of the donor plasmid which might be due to cointegrate formation with pUZ8. Small-scale plasmid DNA preparations from Tp^r transconjugants were also analyzed by restriction endonuclease digestion and agarose gel electrophoresis. Except as mentioned below, plasmid pUZ8 revealed an increase in molecular weight, indicating insertion of the transposon, and contained the expected internal restriction endonuclease fragments of Tn7 or its deleted derivatives.

Removal of DNA from the right-hand end of the transposon, deletions involving the *Bgl*II sites of Tn7 (pHH7001 and pHH7002), abolished transposition (Table 1). Transposition from these plasmids was not restored by complementation in *trans* by pHH7101, which contained all but the left-hand 5 kb of Tn7, which encodes the antibiotic resistance genes. A small number of Tp^r transconjugants were detected, but the analysis of their plasmid DNA revealed that it contained the internal fragments of undeleted Tn7. We concluded that these Tp^r transconjugants resulted from recombination, using a system other than RecA, between the deleted derivatives of Tn7 and the cloned region of pHH7101 (12). Deletion of the *Bgl*II fragment of Tn7 (pHH7001) removed 0.8 kb of DNA inwards from base pair (bp) 42 of the right-hand end of the transposon (19). It seems then that Tn7 requires more than 42 bp of DNA in *cis* at its right-hand end for its transposition. This is more than the 38-bp inverted repeat ends required in *cis* for the transposition of Tn*3* (16).

Deletion of a central region of about 5 kb of Tn7 DNA (pHH7003 ad pHH7004) also abolished transposition. However, transposition at normal frequencies was demonstrated in these plasmids when they were complemented in *trans* by pHH7101. This central region must therefore encode one or more diffusible products essential for transposition and cannot contain a site required in *cis*. It appears that the gene products necessary for transposition are freely diffusible, as is true for Tn*3*, but in contrast to the *cis*-acting proteins involved in the transposition of Tn*5*, Tn*10*, and Tn*903* (16).

Deletion of a smaller fragment from this central 5-kb region, the 2.6-kb *Hin*dIII fragment (pHH7005), also abolished transposition. A putative transposase might be partly

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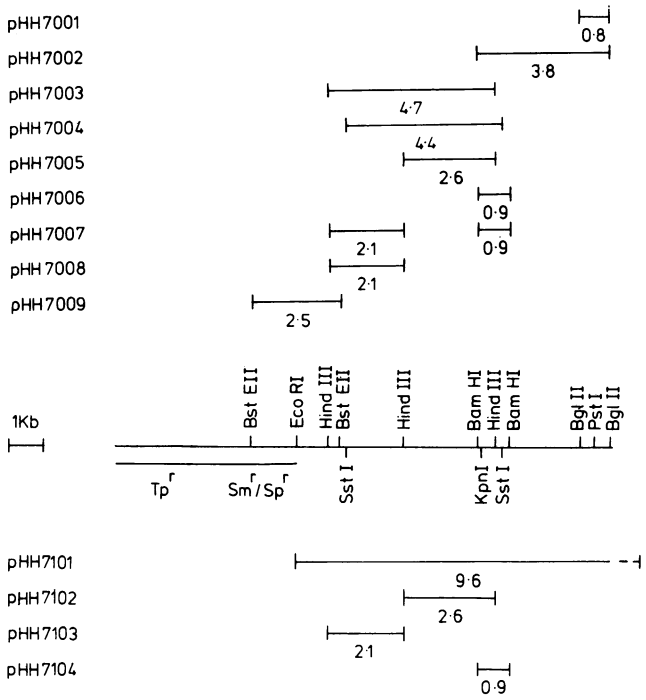

FIG. 1. Restriction endonuclease map of Tn*7*. Bars at top indicate fragments (in kb) of DNA deleted from ColE1::Tn*7* to construct the plasmids listed. Bars at bottom show the fragments (in kb) of Tn*7* cloned into the *Hin*dIII or *Bam*HI site of plasmid pACYC184 to construct the plasmids listed.

encoded by this fragment. Interestingly, transposition from pHH7005 was complemented by pHH7101 only at a low level. This could indicate that deletion of the 2.6-kb *Hin*dIII fragment results in the abnormal expression of a gene product, which reduces the ability of pHH7101 to complement transposition.

Deletion of the 0.9-kb *Bam*HI fragment from Tn*7* (pHH7006) resulted in a 1,000-fold reduction in transposition frequency. As transposition from this plasmid was complemented in *trans* to normal frequencies by pHH7101, the deletion of the *Bam*HI fragment could not have removed or altered a site required in *cis* for transposition. The reduction in transposition frequency could be due to production of a truncated and less efficient transposase, or it could be an effect on the expression of another gene product required for the normal transposition of Tn*7*.

The deletions in plasmid pHH7007 (the 0.9-kb *Bam*HI fragment and the 2.1-kb *Hin*dIII fragment) gave the same results as deletion of the *Bam*HI fragment alone (pHH7006). This seems to indicate that the DNA within the 2.1-kb *Hin*dIII fragment does not encode a product essential for transposition. Paradoxically, deletion of the 2.1-kb *Hin*dIII fragment alone (pHH7008) abolished transposition; the function was complemented in *trans* to normal frequencies by pHH7101 or pHH7103. Thus, the 2.1-kb *Hin*dIII fragment appears to code for a diffusible gene product necessary for transposition when the *Bam*HI fragment is present. Interaction between the gene products encoded by these two regions is implied.

The presence of pHH7103, which contains the 2.1-kb *Hin*dIII fragment of Tn*7*, resulted in 30-fold stimulation of transposition from ColE1::Tn*7*. This fragment must encode a diffusible gene product capable of enhancing transposition.

Deletion of the 2.5-kb *Bst*EII fragment from Tn*7* (pHH7009) did not reduce its frequency of transposition, but resulted in the loss of streptomycin-spectinomycin resistance.

Cointegrate formation between the donor and target plasmids was not detected for any of the deletions in Tn*7*, whether alone or complemented in *trans*. The deletions could not have affected an internal resolution site or resolvase protein like those described for Tn*3* (16).

The results indicate that the mechanism of transposition of Tn*7* is complex, involving several diffusible gene products.

TABLE 1. Effects of deletions in Tn*7* on its frequency of transposition into pUZ8

Plasmid	Frequency of transposition[a] with plasmid tested for complementation:				
	None	pHH7101	pHH7102	pHH7103	pHH7104
ColE1::Tn*7*	3×10^{-3}	4×10^{-3}	6×10^{-3}	9×10^{-2}	3×10^{-3}
pHH7001	$<5 \times 10^{-8}$	1×10^{-7}	—[b]	—	—
pHH7002	$<4 \times 10^{-8}$	3×10^{-7}	$<1 \times 10^{-8}$	—	$<3 \times 10^{-8}$
pHH7003	$<3 \times 10^{-8}$	2×10^{-3}	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$	$<2 \times 10^{-8}$
pHH7004	$<3 \times 10^{-8}$	1×10^{-3}	$<2 \times 10^{-8}$	$<2 \times 10^{-8}$	$<2 \times 10^{-8}$
pHH7005	$<3 \times 10^{-8}$	2×10^{-6}	$<1 \times 10^{-8}$	—	$<6 \times 10^{-8}$
pHH7006	4×10^{-6}	4×10^{-3}	1×10^{-6}	—	4×10^{-6}
pHH7007	3×10^{-6}	2×10^{-3}	2×10^{-6}	2×10^{-7}	1×10^{-6}
pHH7008	$<4 \times 10^{-8}$	2×10^{-3}	—	8×10^{-3}	—
pHH7009	6×10^{-3}	5×10^{-3}	—	8×10^{-2}	—

[a] Values were derived from three or more estimations.
[b] —, Not determined.

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