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8. Homozygous *det2-1* mutant (Col-0) was crossed to either wild-type No-0 or La-*er*. DNAs from F_2 *det2* seedlings were prepared [S. Dellaporta, J. Wood, J. Hicks, *Plant Mol. Biol. Rep.* **1**, 19 (1983)] for simple sequence length polymorphisms (SSLPs) [C. J. Bell and J. R. Ecker, *Genomics* **19**, 137 (1994)] and cleaved amplifed polymorphic sequences (CAPS) analysis [A. Konieczny and F. M. Ausubel, *Plant J.* **4**, 403 (1993)]. Overlapping yeast artificial chromosome (YAC) clones were isolated from three separate YAC libraries of *Arabidopsis* [E. R. Ward and G. C. Jen, *Plant Mol. Biol.* **14**, 561 (1990); J. R. Ecker, *Methods* **1**, 186 (1990); E. Grill and C. Somerville, *Mol. Gen. Genet.* **226**, 484 (1991)]. Fine RFLP analysis was performed with F_2 *det2* plants with recombination break points either in the m323-*DET2* region (68 recombinants, two mapping populations) or in the *DET2*-nga168 interval (31 recombinants), and new CAPS markers were converted directly from YAC insert ends or derived from phage clones of an *Arabidopsis* genomic library isolated with YAC end probes (*9*).
9. Cosmid and phage clones were isolated from two *Arabidopsis* genomic libraries [N. Olszewski, F. Martin, F. Ausubel, *Nucleic Acids Res.* **16**, 10765 (1988); the lambda genomic library was provided by R. W. Davis (Stanford University)] by hybridization with yUP2C12, yUP6B10, YAC end probes, or cosmid-derived probes. Cosmid DNAs were transformed into *det2-1* plants by a modified vacuum infiltration method [N. Bechtold, J. Ellis, G. Pelletier, *C. R. Acad. Sci. Paris* **316**, 1194 (1993); A. F. Bent *et al.*, *Science* **265**, 1856 (1994)] to identify cosmids containing the *DET2* gene.
10. Labeled Eco RI fragments of cosmid 217-61 were used as probes to screen ~2×10^6 clones of an *Arabidopsis* complementary DNA (cDNA) library constructed in lambda ZAPII [J. J. Kieber, M. Rothenburg, G. Roman, K. A. Feldmann, J. R. Ecker, *Cell* **72**, 427 (1993)]. Positive clones were converted to plasmids by in vivo excision according to the manufacturer's protocol (Stratagene) and sequenced with gene-specific primers .
11. The transcribed region of the *DET2* gene was amplified by polymerase chain reaction (PCR) from genomic DNAs of wild-type Col-0 and eight *det2* alleles, subcloned into pGEM-T vector (Promega), and sequenced. To minimize PCR errors, we pooled at least four different clones from two independently amplified fragments for sequencing.
12. Database searches were performed at the U.S. National Center for Biotechnology Information with the BLAST program [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. The sequence alignment and phylogenetic analysis were performed with the Megalign program (DNAStar) by the method of J. Hein [D. G. Higgins and P. M. Sharp, *Comput. Appl. Biosci.* **5**, 151 (1989)].
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17. Seeds were germinated on moist Whatman papers placed on MS medium [0.5× MS salts (Gibco), 1× Gamborg's B5 vitamins (Sigma), 0.8% phytagar (Gibco) and 1% sucrose], pH 5.7, for 2 days and transferred to fresh plates supplemented with various concentrations of auxin (IAA, 0 to 10^{-5} M), brassinolide (0 to 10^{-6} M), and gibberellins (GA1 or GA4, 0 to 10^{-5} M). Hormones were sterile-filtered into the cooling MS medium. For dark-grown seedlings, seeds were exposed to 2-hour light treatment before their plates were wrapped with three layers of aluminum foil, and the seedlings were transferred under a green safe-light. The hypocotyl lengths of 10-day-old etiolated seedlings and 12-day-old light-grown wild-type plants were measured.
18. Single-letter abbreviation for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, The; V, Val; W, Trp; and Y, Tyr.

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Switching from Cut-and-Paste to Replicative Tn7 Transposition

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The bacterial transposon Tn7 usually moves through a cut-and-paste mechanism whereby the transposon is excised from a donor site and joined to a target site to form a simple insertion. The transposon was converted to a replicative element that generated plasmid fusions in vitro and cointegrate products in vivo. This switch was a consequence of the separation of 5′- and 3′-end processing reactions of Tn7 transposition as demonstrated by the consequences of a single amino acid alteration in an element-encoded protein essential for normal cut-and-paste transposition. The mutation specifically blocked cleavage of the 5′ strand at each transposon end without disturbing the breakage and joining on the 3′ strand, producing a fusion (the Shapiro Intermediate) that resulted in replicative transposition. The ability of Tn7 recombination products to serve as substrates for both the limited gap repair required to complete cut-and-paste transposition and the extensive DNA replication involved in cointegrate formation suggests a remarkable plasticity in Tn7's recruitment of host repair and replication functions.

Transposable elements are DNA segments that can move from one DNA site to another in the absence of homology between the two sites. Many elements, including the bacterial transposon Tn7, move through a cut-and-paste mechanism in which the transposon is first excised from the donor site by double-strand breaks at each end of the transposon and is then joined to the target site (*1*–*3*) (Fig. 1). This joining occurs through the linkage of the 3′ ends of the transposon to staggered positions on the top and bottom strands of the target DNA, resulting in small (several nucleotide) gaps flanking the newly inserted transposon. Repair of these gaps by host functions generates the small flanking direct repeats characteristic of transpositional recombination.

Other transposons, including bacteriophage Mu and Tn3, can move via a replicative pathway which involves extensive DNA replication (*1*, *2*). In Mu, only one strand at each end of the element is cut, exposing the 3′ transposon end. Transfer of this end to the target DNA results in a fusion product between the donor and target molecules termed a Shapiro intermediate or strand transfer intermediate, in which one strand at each end of the transposon remains joined to the donor DNA, and the other is joined to the target DNA (*4*, *5*). Repair of this transposition product by host functions involves much more extensive DNA replication than the repair of simple insertions (up to tens of kilobases compared to several nucleotides) and generates a circular cointegrate molecule in which the donor and target sequences are joined by two copies of the transposon.

A fundamental difference between the cut-and-paste and replicative transposition pathways lies in the cleavage of the 5′ ends of the transposon (*2*). In standard Tn7 recombination the break is double-stranded, that is, both 5′ and 3′ strands are cleaved in the cut-and-paste transposition. We now show that, with a single point mutation in a Tn7-encoded protein, 5′ end cleavage is blocked and the transposition mechanism is switched; Tn7 now transposes replicatively to produce a Shapiro intermediate, which can be subsequently processed into a cointegrate transposition product.

Tn7 transposition requires an elaborate array of Tn7-encoded proteins: TnsA, TnsB, TnsC, TnsD, and TnsE (*3*, *9*, *10*). TnsA and TnsB constitute the heart of the transposition machinery that executes the DNA breakage and joining reactions that

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underlie recombination (*11–13*). The ends of the transposon are specifically recognized by TnsB (*14, 15*); TnsA is probably recruited to the ends through interactions with TnsB (*8, 11*). The remaining Tns proteins modulate the activity of TnsA and TnsB and direct transposition to two alternative types of targets (*3, 9, 10, 16*). Insertions into all targets require the adenosine triphosphatase TnsC which activates the TnsA+TnsB machinery assembled at the ends of the element (*11, 16*). The TnsD protein binds to a sequence on the bacterial chromosome *attTn7* and, through TnsC, directs transposition to this site (*8*). Alternatively, TnsE directs insertions into other non-*attTn7* targets.

We examined the activity of a TnsA mutant generated through site-specific mutagenesis (*17*), an alanine (D) substitution for aspartate (A) at position 114 (D114A), in an in vitro reconstituted Tns(ABCD) transposition system (Fig. 2). Purified Tns proteins (*18*), adenosine triphosphate (ATP), and Mg^{2+} were incubated with a donor plasmid containing a mini-Tn7 element, and a target plasmid containing *attTn7* (*19*). No reaction was observed without TnsA (lane 1). With wild-type TnsA, the principal reaction product was a simple insertion (Fig. 2, lane 2). Recombination intermediates of Tn7 cut-and-paste transposition were visible, namely, donor molecules with double-stranded breaks (DSBs) at either transposon end. Also present were by-products in which only the cleaved transposon end of a DSB was joined to the target, forming a double-strand break single-end join (DSB-SEJ).

Unlike the reaction with wild-type TnsA, reactions containing D114A TnsA generated a previously unobserved recombination product, the fusion product (FP); no products indicative of cut-and-paste transposition (DSBs or simple insertions) were observed (Fig. 2, lane 13). As established by electron microscopy and denaturing gel electrophoresis (Fig. 3 and 4), this new product is a fusion of the donor and target plasmids joined through a copy of the transposon (Fig. 3), structurally equivalent to the Shapiro intermediate of replicative Mu transposition (*4, 5*). Although 5′ end cleavage was totally blocked by the D114A TnsA mutation, this alteration had not obviously affected the 3′ end cleavage and strand transfer activities. The only difference between the Tn7 fusion product and a simple insertion is the lack of cleavage at the 5′ transposon ends.

Reactions containing mixtures of wild-type and D114A TnsA (Fig. 2, lanes 8 to 12) produced two other products that are also fusions of the donor and target plasmids. However, one "arm" of the donor molecule remained attached to the transposon, but the other had been released through a double-stranded break; that is, 3′ cleavage and transfer occurred at both transposon ends, but 5′ cleavage was conducted at only one end (*20*). In one new species of lower mobility, the right end of the transposon was still attached to the donor DNA, but the left end (L) has been

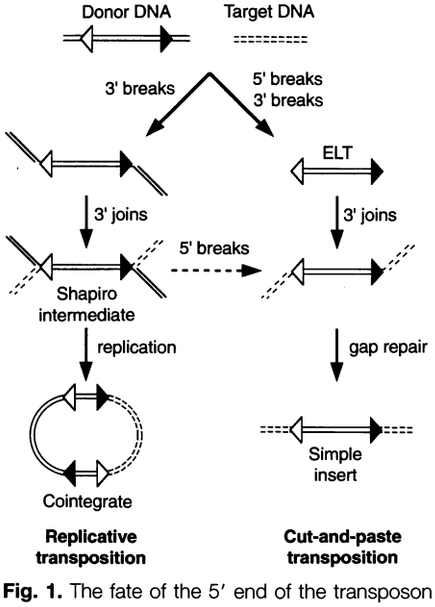

Fig. 1. The fate of the 5′ end of the transposon (cleaved, or remaining joined to the donor molecule) differs between the two common transposition pathways. Donor DNA (solid line) contains the transposon flanked by triangles representing the cis-acting recombination sequences; target DNA (dashed line). Cut-and-paste Tn7 transposition proceeds through excised linear transposon (ELT) intermediates to the final product, the simple insertion. Replicative transposition generates the Shapiro intermediate, which is replicated to form a cointegrate molecule. In some systems, a subsequent site-specific recombination reaction resolves the cointegrate, regenerating the donor molecule and generating a target molecule now containing a copy of the transposon (*6*).

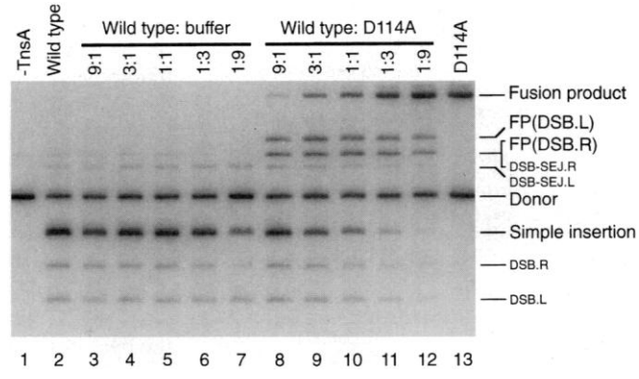

Fig. 2. Wild-type and D114A TnsA are tested in vitro. After the transposition reaction, a third of the purified DNA was digested with Nde I (which cuts asymmetrically once each in the donor and target molecules), placed on an 0.6% agarose gel, transferred to nitrocellulose, and then probed for the presence of the mini-Tn7 element. Unreacted donor runs at 5926 bp; simple insertion, 4815 bp; DSB.R, 4089 bp; DSB.L, 3462 bp. Other noted products are jointed molecules that are anomalously slow for their molecular weight. Lanes 2 through 7: a titration of wild-type TnsA. Lanes 8 through 12: a mixture of wild-type and D114A TnsA, with the total TnsA concentration was held constant.

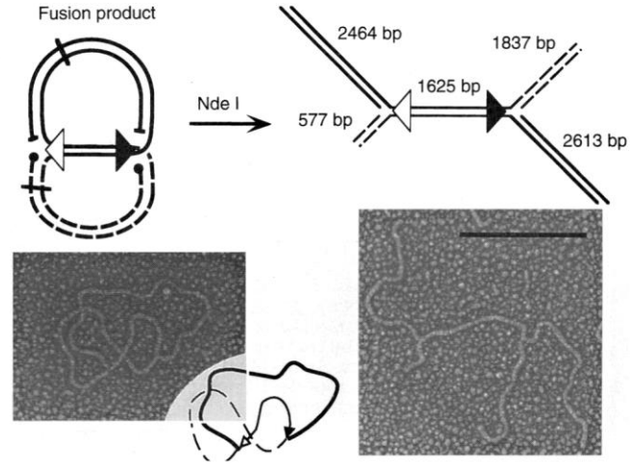

Fig. 3. Representative electron micrographs (EM) of the D114A TnsA FP. DNA was extracted from in vitro reactions and applied to EM grids (*30*). The FP is displayed diagramatically both undigested and digested with Nde I (top). The 3′ ends of the transposon are joined to the target DNA (dashed line) while the 5′ ends of the transposon remain joined to the donor DNA (solid line) yielding a plasmid fusion. Undigested FPs were visible usually as small supercoiled knots of DNA. Occasionally, however, a relaxed molecule of the characteristic theta shape was encountered (*31*). Joints are distinguished from overlying DNA strands in the accompanying trace; donor backbone, bold line; transposon, bounded by triangles; target DNA, dashed line. Nde I cuts the FP as indicated, releasing supercoils and yielding a joint molecule with five characteristic length segments. Tracing indicated that their lengths were proportional to the expected values to within 5%. The standard bar is 0.5 μm.

cleaved by a DSB [FP(DSB.L]. The other new species, in which the right end has been cleaved, comigrated with DSB-SEJ.R. These alternative products imply that at least two TnsA proteins take part in each recombination complex. None of these above described products were the result of simple titration of TnsA (Fig. 2, lanes 2 to 7).

These experiments show that the DNA breakage and joining activities at the 3′ ends were maintained with the D114A TnsA mutation. Yet these 3′ reactions are still dependent on the presence of TnsA; no breakage and joining products were observed when TnsA was simply omitted from the reaction. Thus, at least two transposition functions, 5′ end cleavage and the 3′ end activities, depend on TnsA.

The consequence of the D114A mutation of TnsA demonstrates a specific separation of the 5′ and 3′ recombination activities that underlie Tn7 recombination, an effect previously unobserved (so far as we know) in transposition systems as the result of transposase alterations. Other than this single protein mutation, no change was introduced into the in vitro transposition reaction to effect the switch from cut-and-paste to replicative Tn7 transposition. Therefore both of these pathways probably result from the same core element-encoded transposition machinery, Tns(ABCD).

Whether the D114A TnsA-mediated Tn7 FP can be generated in vivo was addressed as follows. In the Shapiro intermediate of replicative bacteriophage Mu transposition (*2*, *4*, *5*), the 3′ hydroxyl groups that flank the transposon prime DNA synthesis into the transposon portion of the molecule to generate a cointegrate (Fig. 1).

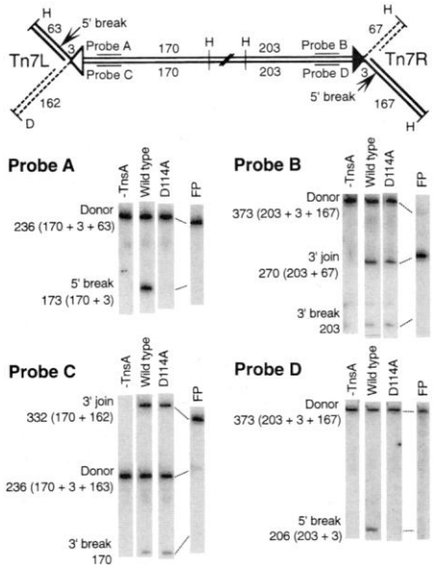

Fig. 4. Denaturing gel analysis of recombination products. The products of in vitro reactions with wild-type and D114A TnsA, and also gel-purified FPs, were examined at the nucleotide level. A schematic of the FP, displayed above the blots, shows the transposon portion of the molecule bounded by arrowheads indicating the left and right ends; the donor backbone portion is in boldface; the target DNA portion is shown in dashed lines. The distance in nucleotides of each restriction site (D, Dra I; H, Hinc II) from the end of the transposon is indicated. While the 3′ breaks at the ends of the transposon are flush with the ends of the element, the 5′ breaks are displaced by three nucleotides (*7*, *12*). The ^{32}P-labeled oligonucleotides used as strand-specific probes for the Southern (DNA) blot are indicated hybridizing specifically to their respective strands (*12*). Below, substrates and products are indicated to the left of each blot, along with their expected molecular lengths expressed as a sum of the distances in the schematic. The 3′ breaks are visible at low levels with probes B and D; these products are rapidly processed to 3′ joins. The D114A TnsA reactions are similar to those of wild type as assayed by the 3′ strand probes (probes B and C, lane 3); thus 3′ breaks and joins proceed. However, there is no D114A TnsA 5′ end cleavage product (probes A and D); 5′ end cleavage is blocked by the D114A mutation. In the gel-purified FP, the 3′ ends of the transposon have been efficiently cleaved and transferred to the target (probes B and C). However, the 5′ ends are still attached to the donor molecule; the product runs as the substrate on the 5′ end–specific blots (probes A and D).

Table 1. Replicative Tn7 transposition with D114A TnsA. A mating-out assay was used to examine the effect of TnsA mutants in vivo (*22*). The source of TnsA is indicated to the left. Cointegrates are not the product of homologous recombination between a simple insertion product and the donor molecule; such events occur at a level comparable to that of background Tn7-independent recombination observed with a deletion of TnsA (line 2), or without Tns functions (*20*, *21*). *N*, number of colonies examined in determining the class of transposition product.

	Transposition frequency (Km^r)	Simple insertion fraction (Km^rCm^s)		Cointegration fraction (Km^rCm^r)	
		Percent	*N*	Percent	*N*
Wild type	8×10^{-4}	>99.99		<0.01*	
−TnsA	$<4 \times 10^{-8}$	<3	0	>97	27
D114A	5×10^{-4}	18	80	82	357

*This measurement of the cointegration frequency with wild-type TnsA was made possible by directly plating the mating mixture onto media with Nal and Cm.

Consistent with the DSBs intrinsic to cut-and-paste transposition (*7*, *8*), no wild-type Tn7-mediated cointegrate formation has been observed above background (*21*; and data presented below).

Our transposition assay in vivo relies on the genetic consequences of transposition (*22*). If a gene for kanomycin resistance (Km^r) is placed inside the transposon and a gene for chloramphenicol resistance (Cm^r) is placed in the backbone portion of the donor molecule, a cointegrate would carry both Km^r and Cm^r, whereas a simple insertion would carry only Km^r. The target is a mobilizable plasmid, a derivative of the F mating factor, allowing for the separation of product molecules from donor molecules through conjugation.

In contrast to wild-type TnsA where Tn7-mediated cointegration was not detected above background (*21*), the D114A mutant of TnsA supported the production of cointegrate molecules (82% of all transposition events) (Table 1). Thus, the FP observed in vitro was also made in vivo, and was subsequently processed into a replicative transposition product. The frequency of D114A TnsA-supported transposition, that is, joining of the transposon to the target plasmid, was comparable to that of wild-type TnsA.

With D114A TnsA, most of the transposition events yielded cointegrate molecules, but 18% were simple insertions. Yet, no simple insertions were observed with D114A TnsA in vitro. Whether these in vivo simple insertions are generated by (i) partial suppression of the phenotype of the D114A TnsA in vivo, so that it executes some 5′ end cleavage, or (ii) a host enzyme nicking the FP before replication begins, such that the donor portion of the molecule is released, producing a simple insertion is not known. However, the latter host mechanism is postulated for the production of simple insertions during lysogenic bacteriophage Mu transposition (*2*, *5*, *23*).

The question also arises as to how Tn7 may alternatively recruit two likely different host functions—one responsible for the minimal gap repair of cut-and-paste transposition and another for the extensive replication required for cointegrate formation (*7*, *8*, *24*). The recruitment of host replication functions during replicative bacteriophage Mu transposition is an elaborate process. The Mu transposase remains tightly associated with its Shapiro intermediate and at least three distinct host factors contribute to the recruitment of host replication functions (*25*). It is not unreasonable that Tn7 transposition proteins may also remain tightly associated with product DNA molecules and that the specialized recruitment of host functions to such a nucleoprotein complex may not be restricted to bacteriophage Mu. Thus,

Tn7 provides a means to investigate how distinctive transposition products and their associated proteins may alternatively recruit the likely different host machines that mediate the gap repair of simple insertions and the extensive replication of Shapiro intermediates.

The discovery within Tn7 of a simple toggle, through point mutation, between cut-and-paste and replicative modes of transposition lends a biochemical vantage point to the discrimination of these two different mechanisms (Fig. 1). All transposition systems that have been studied with regard to mechanism share the same chemistry of 3′ end breakage and strand transfer (*2*); nevertheless, although cut-and-paste transposons cleave at the 5′ end, replicative transposons do not. The lack of 5′ end cleavage during replicative Mu and (likely) Tn3 transposition generates the Shapiro intermediate, which can then be replicatively processed into a cointegrate molecule (*2, 5*). In contrast, DSBs (cuts on both 5′ and 3′ ends) occur before 3′ strand transfer with cut-and-paste transposons (*7, 8, 26, 27*).

We have shown that the core Tn7-encoded recombination machinery contains all the active sites necessary for the breakage and joining chemistry of both the cut-and-paste and replicative modes of transposition. In vitro Mu simple insertion, like replicative Mu transposition, is thought to proceed through the Shapiro intermediate, but with 5′ end cleavage occurring after the 3′ strand transfer events (*2, 5, 23*). In contrast to Tn7, the Mu 5′ cleavage activity appears to be provided by host functions rather than by the alternative action of its transposase (*5*). The 5′ and 3′ end cleavage activities of cut-and-paste transposition, however, need not be in separate proteins. In the well-characterized Tn10 transposition system, Tn10 transposase, which contains a single gene product, is sufficient to catalyze DSBs and 3′ strand transfer in vitro (*26–28*). But unlike Tn7, the generation of Tn10 Shapiro intermediates in vitro has not been demonstrated. Indeed, the suppression of 5′ cleavage during Tn10 transposition may not be possible; Tn10 requires DSBs at both ends of the element before the engagement of target DNA (*26*).

Some insertion sequences, such as IS903 and IS1, predominantly form direct simple insertions but also appear to form replicative cointegrates at a significant rate (*29*). It is possible that these elements are functional through a mechanism similar to that of Tn7, where two different transposition products are formed depending on the alternative action of the element-encoded transposase. Alternatively, IS903 and IS1 may employ a Mu-like mechanism, efficiently forming Shapiro intermediates, with the ultimate fate of the transposition product being dependent on the 5′ end cleavage activity of a host factor.

The ease with which this switch between the simple insertion and cointegrate forming pathways in Tn7 is possible—a single amino acid change in one transposition protein—introduces the possibility that there may be alternative conditions in vivo which would allow Tn7 to interchange naturally between these two mechanisms. Just as Tn7 can use distinct classes of target sites (*3, 9, 10*), this transposon may also be capable of switching mechanistic pathways to generate alternate transposition products that are potentially beneficial to the transposon under different cellular conditions.

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17. D114 of TnsA corresponds to a conserved position in this protein's putative DD-35-E motif, a domain common to many transposases and implicated as the catalytic site (*13*). The mutation was introduced into the TnsA gene by a polymerase chain reaction (PCR)–based procedure. Oligonucleotides were synthesized such that a PCR reaction with pEM279 [a derivative of pGST-A (*8*)] as a template would generate a small fragment containing the point mutation flanked by two cloning sites. The product of the PCR was isolated and treated with appropriate restriction enzymes. The mutagenic insert was gel-purified and then ligated into pEM279. Unless otherwise noted, all molecular and genetic protocols were from J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual*, C. Nolan Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989).
18. TnsA was expressed as a fusion with glutathione-S-transferase by a modified version of the protocol of Bainton *et al.* (*8*). Thrombin was added to the fusion protein while still bound to the glutathione matrix (5 units of thrombin per 1 ml of suspension incubated at room temperature for 40 min). The TnsA-containing supernatant from low speed centrifugation was dialyzed against TnsA storage buffer [25 mM Hepes, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol (DTT), and 5% glycerol]. TnsB and TnsD were prepared as His^6-fusions isolated with a Ni^{2+}-coordinated column (*12, 13*) (P. L. Sharpe and N. L. Craig, unpublished data). TnsC was prepared as described by P. Gamas and N. L. Craig [*Nucleic Acids Res.* **20**, 2525 (1992)].
19. In vitro reactions (*8, 12*) were prepared on ice. A target complex was formed by incubating the target DNA (pRM2, 3.2 kb, containing *attTn7*), TnsC, TnsD, and ATP with other buffer components. After 20 min at 30°C, the donor DNA (pEMΔ, 5.9 kb, containing a 1.6-kb mini-Tn7), TnsA, TnsB, and magnesium acetate were sequentially added, and the reaction was allowed to continue for 20 min. Final reaction conditions in 100-μl total volume were 0.25 nM pEMΔ, 2.5 nM pRM2, 26 mM Hepes (pH 8), 4.5 mM tris-Cl (pH 7.5), 2.1 mM DTT, 15 mM magnesium acetate, 2 mM ATP, tRNA at 100 μg/ml, bovine serum albumin at 50 μg/ml, 44 μM EDTA, 120 μM $MgCl_2$, 14 mM NaCl, 21 mM KCl, 120 μM CHAPS, 4 μM PMSF, 1.2% glycerol, 24 nM TnsA, 3 nM TnsB, 8 nM TnsC, and 6 nM TnsD. Reactions were stopped with 25 mM EDTA.
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22. A DNA fragment from pMIM-1 (*7*) containing the left end of Tn7 and Km^r was inserted into pCW4 (*10*) which contains the right half of Tn7, including all five Tns genes to make pEM287, which now contains a Km^r mini-Tn7 element. The tetracycline resistance gene outside the transposon was replaced with a chloramphenicol resistance gene (Cm^r) to make pEM289, the donor plasmid for the in vivo mating-out assay. The central 85% of TnsA from the BstX I to Bgl II sites, were deleted to generate pEM292. The D114A point mutation of TnsA was introduced into the mini-Tn7 element of pEM289 on a cassette flanked by the Msc I and Bgl II sites of TnsA. Our assay for cointegration was a standard mating-out experiment (*21*). The experimental donor plasmid replaced pCW4 in LA457 (*recA*$^-$, blocked chromosomal *attTn7*). The target plasmid [pOX-Gen, a derivative of the F mating factor carrying gentamycin resistance (Gm^r)] was mated out into an F^- naladixic acid resistant (Nal^r) strain (CW51). The transposition frequency was calculated as the fraction of transconjugants that carry resistance to Km: Nal^r-Km^r/$Nal^r Gm^r$. The $Nal^r Km^r$ transconjugants were patched to media containing Cm to determine whether the transposition event was due to a simple insertion event (Cm^s) or a cointegration event (Cm^r). These in vivo experiments were conducted with the Tns(ABCE) pathway of transposition; consistent results were obtained with the Tns(ABCD) pathway.
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