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Orientation of IS*50* Transposase Gene and IS*50* Transposition

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Reversal of transposase gene orientation with respect to the nonidentical ends of IS*50* strongly decreased IS*50* transposition in both Dam$^-$ and Dam$^+$ hosts. In either orientation, IS*50* transposase expression was unaffected. These effects were independent of the surrounding DNA context. This shows that the efficiency of IS*50* transposition is dependent on transposase gene orientation. The transposition frequencies of transposons utilizing inverted IS*50* inside ends (IE), IE-IE transposons, were lower than either outside end (OE)-IE or OE-OE transposons.

Tn*5* is a composite of two inverted IS*50* sequences that flank a unique DNA sequence encoding several antibiotic resistances (10). Insertion sequence IS*50* is defined by nonidentical end sequences, the inside end (IE) and the outside end (OE), and encodes the transposase protein required for its own transposition (3, 6, 13). Each end is named for its proximity to the unique region of transposon Tn*5*. IS*50* encodes two proteins from the same reading frame: the P1 transposase and the P2 inhibitor (3, 5, 13). The P1 transposase is 55 amino acids longer than the P2 inhibitor, and the mRNA which encodes P1 is transcribed from a different promoter (7). IS*50* transposition is increased approximately 1,000-fold in a Dam$^-$ host compared with transposition in an isogenic Dam$^+$ host (9). It is presumed that approximately a 10-fold transposition increase in Dam$^-$ hosts is due to the methylation state of two GATC *dam* methylase recognition sites in the −10 region of the P1 promoter (11, 15). When these sites are unmethylated, the level of transposase expression increases. There is approximately a 100-fold contribution to the transposition increase when two GATC *dam* methylation sites in the IE are unmethylated (9). The OE does not contain GATC *dam* methylation sites. These and other properties of IS*50* and Tn*5* have recently been reviewed (1).

In wild-type IS*50*, the P1 promoter is found proximal to the OE with the carboxy-terminal coding regions for the P1 and P2 genes proximal to the IE. Since the P1 transposase is considered a *cis*-acting protein, it was thought that the transposase gene orientation with respect to its putative recognition sites, the IS*50* ends, may influence transposition efficiency (3, 4, 6, 16). To test this theory, the following experiments were performed.

Four plasmids were constructed, each containing IS*50* end sequences that flank the genes encoding the IS*50* P1 and P2 proteins (Fig. 1). The OE-OE transposons on plasmids pRZ1494 and pRZ1495 are identical to each other, as are the OE-IE transposons on plasmids pRZ1497 and pRZ1498. However, the orientation of each identical transposon in the plasmid is reversed. OE-IE transposons on plasmids pRZ1499 and pRZ1470 are also identical with reversed orientations on the ColE1 plasmid. The internal configurations of the transposons in pRZ1499 and pRZ1470 are different from the configurations of the transposons in pRZ1497 and pRZ1498. The IS*50* ends were sequenced to ensure that no mistakes were made in plasmid construction. The levels of transposition from the transposon on each plasmid were determined by two independent methods (5, 8, 9). Transposition data from these plasmids allow a determination of the effects that a change in transposase gene orientation with respect to the IS*50* ends would have on transposition.

Identical transposons with the same orientation of transposase gene relative to the ends have the same transposition frequency independent of the orientation of the transposon to the plasmid origin of replication (Fig. 1). When the orientation of the transposase gene with respect to the ends is reversed, however, the level of transposition changes approximately 10-fold. This is true in either a Dam$^+$ or a Dam$^-$ host. The most efficient configuration for transposition is the wild-type case, in which the P1 promoter is proximal to the OE. These results agree with those of Dodson and Berg (2) for the Dam$^+$ case, although those authors did not observe as large a relative increase in transposition for transposons with the preferred transposase gene orientation in Dam$^-$ hosts.

In order to determine the efficiency of IE usage in transposition, plasmids pRZ1471 and pRZ1472, in which the transposase gene is flanked by two inverted IEs, were made. The transposons in these constructs are identical, although the orientation of the transposase genes with respect to plasmid sequences is reversed. From the data in Fig. 1, it is apparent that IE-IE transposition was not as high as that seen by Dodson and Berg (2). We have no explanation for this difference.

To determine whether the proximity of the different ends to the P1 promoter could affect the level of IS*50* protein expression, the plasmids in Fig. 2 were made. In pRZ1452 and pRZ1453, the P1 promoter is proximal to the OE and IE, respectively, and both P1 and P2 β-galactosidase fusion proteins are expressed from each plasmid (7). In pRZ1454 and pRZ1455, the P1 promoter is proximal to the OE and IE, respectively, and only a P1 β-galactosidase fusion protein is expressed from each plasmid (7). The level of fusion protein expression was measured by standard β-galactosidase assays (12). In Dam$^+$ and Dam$^-$ hosts, neither the level of expression of the P1 and P2 proteins together nor the level of expression of the P1 fusion protein alone is affected by changing the IS*50* end proximal to the P1 promoter (Fig. 2).

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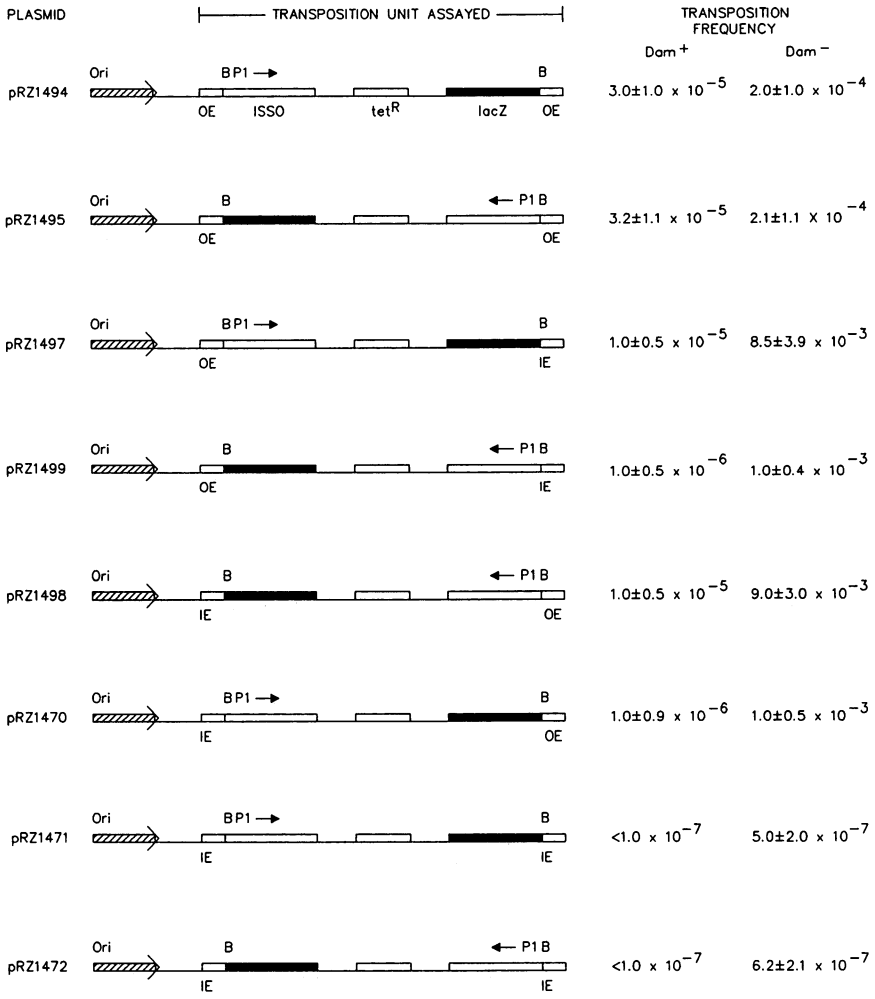

FIG. 1. Representations of the transposons used for this study. All are identical in size (12 kilobase pairs) and contain the same sequences. However, their internal configurations and orientations with respect to the plasmid origin of replication (Ori) are different. The plasmid sequences are 6 kilobase pairs long. The figures are not drawn to scale. OE, 23-base-pair IS*50* OE (CTGACTCTTATACACAAGTAGCG); IE, IS*50* IE (CTGTCTCTTGATCAGATCTTGATCCCC); B, *Bam*HI restriction sites used to reverse the orientation of the transposase promoter (P1) with respect to the IS*50* ends. The shaded area of the transposon represents the *lacZ* gene used for assaying transposition by the papillation method (8, 9). Transposition frequencies were determined in isogenic strains by the mating-out assay (5, 9). Transposition frequencies are the ratios of exconjugant p*OX38*-gen episomes that obtain the drug resistance of the transposon to the number of exconjugants that do not. The tetracycline A resistance gene was used as the transposon marker (tet^R) (9).

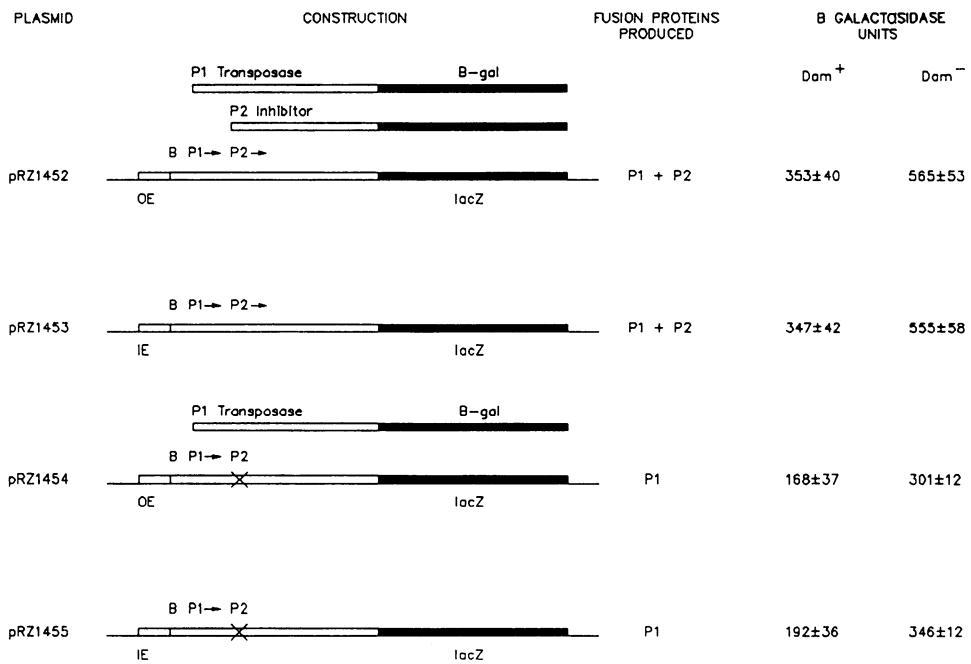

FIG. 2. Representations of the genes encoding IS*50* protein P1 (transposase) and P2 (inhibitor) fusions to β-galactosidase (B-gal). In plasmids pRZ1452 and pRZ1453, β-galactosidase protein fusions of both P1 and P2 proteins are expressed (7, 15). In pRZ1452, and P1 and P2 promoters are proximal to the IS*50* OE, while in pRZ1453 they are proximal to the IE. Plasmids pRZ1454 and pRZ1455, however, express only a β-galactosidase protein fusion of the P1 protein (7, 15). In pRZ1454, the promoter is proximal to the OE, while in pRZ1455, the P1 promoter is proximal to the IE.

These results suggest that a preferred order exists for IS*50* end utilization during the transposition process. This order is dependent on the proximity and orientation of the transposase gene relative to the IS*50* ends as well as on the methylation state of the IE. In all cases, on OE proximal to the P1 promoter is preferred. With this condition met, transposition is most efficient with a distal unmethylated IE, less efficient with a distal OE, and least efficient with distal methylated IE. The reason for the role of transposase gene orientation in IS*50* transposition is unknown but may have to do with the *cis* activity of the P1 transposase protein. The reason for the enhanced frequency of IS*50* (OE-IE) Dam$^-$ transposition compared with Tn*5* (OE-OE) Dam$^-$ transposition is also unknown. It is possible that the OE-associated transposition apparatus has an intrinsic preference for the unmethylated IE and/or that the preferred collection of host proteins in transposition is a combination of those which interact with the OE and those which interact with the IE.

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