Copyright © 1986 by the Genetics Society of America

LOCALIZATION OF ACTION OF THE IS*50*-ENCODED TRANSPOSASE PROTEIN

SUHAS H. PHADNIS, CHIHIRO SASAKAWA[1] AND DOUGLAS E. BERG

Department of Microbiology and Immunology and Genetics, Box 8093, Washington University School of Medicine, St. Louis, Missouri 63110

Manuscript received June 10, 1985
Revised copy accepted October 26, 1985

ABSTRACT

The movement of the bacterial insertion sequence IS*50* and of composite elements containing direct terminal repeats of IS*50* involves the two ends of IS*50*, designated O (outside) and I (inside), which are weakly matched in DNA sequence, and an IS*50* encoded protein, transposase, which recognizes the O and I ends and acts preferentially in *cis*. Previous data had suggested that, initially, transposase interacts preferentially with the O end sequence and then, in a second step, with either an O or an I end. To better understand the *cis* action of transposase and how IS*50* ends are selected, we generated a series of composite transposons which contain direct repeats of IS*50* elements. In each transposon, one IS*50* element encoded transposase (tnp^+), and the other contained a null (tnp^-) allele. In each of the five sets of composite transposons studied, the transposon for which the tnp^+ IS*50* element contained its O end was more active than a complementary transposon for which the tnp^- IS*50* element contained its O end. This pattern of O end use suggests models in which the *cis* action of transposase and its choice of ends is determined by protein tracking along DNA molecules.

THE movement of transposable elements to new sites in a genome occurs without extensive DNA homology and involves recognition of the element ends by specific proteins called transposases. Complementation tests have shown that some of the bacterial transposases act preferentially in *cis* when selecting transposable element ends (ISBERG and SYVANEN 1981; BERG *et al.* 1982a; MACHIDA *et al.* 1982; MORISATO *et al.* 1984; PATO and REICH 1984). IS*50*, the element present in the Kan^r transposon Tn*5* (for review, BERG and BERG 1983), and the focus of the experiments presented here, encodes such a *cis*-acting transposase (ISBERG and SYVANEN 1981; BERG *et al.* 1982a). The sequences needed for its efficient transposition, designated O (outside) and I (inside), extend over the first 19 bp at each end of the element (SASAKAWA,

Abbreviations used in text: *amp*, *tet* and *kan*, genes encoding resistances to ampicillin, tetracycline and kanamycin, respectively; superscript r, resistance; superscript s, sensitivity; *tnp*, the gene encoding transposase; O and I, the 19-bp segments at each end of the insertion element IS*50*, designated with regard to their positions in transposon Tn*5*, which contains inverted terminal repeats of IS*50* (outside and inside, respectively); bp, base pair(s); kb, kilobase pairs.

[1] Present address: Institute of Medical Science, University of Tokyo, Tokyo 108, Japan.

CARLE and BERG 1983; SASAKAWA *et al.* 1985; JOHNSON and REZNIKOFF 1983) and are matched in DNA sequence at just 12 of 19 positions (BERG *et al.* 1982b). Studies of several IS*50*-based composite transposons had shown that segments bounded by pairs of IS*50* O ends, or one O end plus one I end, move with similar efficiencies, but that segments bounded by pairs of I ends move <1% as frequently. This result had suggested that, initially, transposase interacts preferentially with an O end and then, in a second step, with either O or I (BERG *et al.* 1982b; SASAKAWA and BERG 1982; ISBERG and SYVANEN 1981).

The complementation tests reported here indicate that when two O ends are present in a single DNA molecule, but only one is near a functional transposase (*tnp*) gene, the O end closest to the gene in which transposase is encoded is most active in transposition. This outcome supports a model (BERG *et al.* 1982a; MORISATO *et al.* 1984) in which transposase generally associates with DNA during its synthesis and then tracks along the DNA molecule in search of IS*50* ends.

MATERIALS AND METHODS

Media, and molecular genetic techniques for bacterial and phage growth, plasmid DNA extractions, restriction endonuclease digestion, ligations and linker insertion mutagenesis have been described earlier (MANIATIS, FRITSCH and SAMBROOK 1982; SASAKAWA and BERG 1982; SASAKAWA, CARLE and BERG 1983).

The strains of *Escherichia coli* K-12 used in this work were DB104 [F^- *supE* Δ*proB-lac* Δ*trpE5* *str*^{r}, lysogenic for λ *b515 b519 xisam6 c*I857 *nin*5 *Sam7* (EGNER and BERG 1981)]; DB973 [*supE*$^+$ (BERG *et al.* 1975)] to determine titers of plaque forming phage in lysates; DB1873 [F^- *recA1 supE*$^+$ Δ*trpE5* Δ*proB-lac str*^{r}, lysogenic for λ *Dam15 b515 b519 intam29 red*Δ*15 imm21cI*^{ts} *Sam7* (HIRSCHEL and BERG 1982)]; DB1891 [F^- *hfl-1 recA1 supE*$^+$ Δ*trpE5* (HIRSCHEL and BERG 1982)], which, because of the *hfl-1* allele, can be lysogenized by phage λ after infection at a multiplicity of one phage per cell (otherwise a higher multiplicity of infection is needed; WULFF and ROSENBERG 1983).

Plasmid pBRG700 (Amp^r) and plasmid pBRG701 (Tet^r) are derivatives of pBR322 containing IS*50*R (Figure 1; SASAKAWA, CARLE and BERG 1983). The *tnp* gene, including its promoter, extends from positions 40 (near the O end) to 1524 (11 bp from the I end) (AUERSWALD, LUDWIG and SCHALLER 1980; JOHNSON and REZNIKOFF 1981, 1984; LOWE and BERG 1983).

RESULTS AND DISCUSSION

To assess whether recognition of an IS*50* O end depends on its position relative to the *tnp* gene, we carried out complementation tests, diagramed in Figure 2. These tests involved pairs of linked IS*50* elements, only one of which contained a functional *tnp*$^+$ gene. Null mutations in *tnp* were generated by insertion of synthetic oligonucleotides (8-bp *Bam*HI linkers) at each of five restriction sites (*Hpa*I, *Xho*I, *Pst*I, *Hin*dIII and *Pvu*II) in the IS*50* elements of the monomeric Amp^r or Tet^r plasmids, diagramed in Figure 1. Complementary monomeric plasmids were then spliced together *in vitro* to generate heterodimeric plasmids containing *tnp*$^+$ and *tnp*$^-$ IS*50* elements as direct repeats bracketing the *amp* and *tet* resistance genes. Each heterodimeric plasmid contained a pair of complementary transposons. In the plasmid designated type I in

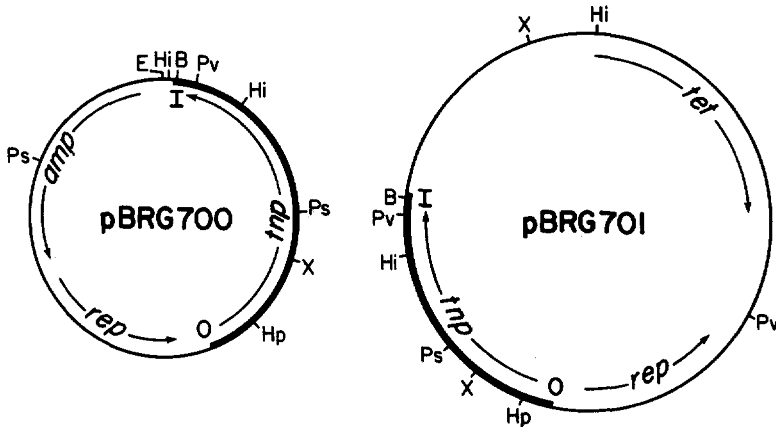

FIGURE 1.—Maps of the pBR322-derived plasmids pBRG700 (3.6 kb) and pBRG701 (6 kb). Thickened lines indicate the 1534-bp IS*50*R element of transposon Tn*5* (hereafter called IS*50*). O and I correspond to the outside and inside ends of IS*50* in Tn*5*. The restriction sites relevant to the present experiments are designated as follows: B, *Bgl*II; E, *Eco*RI; Hi, *Hin*dIII; Hp, *Hpa*I; Ps, *Pst*I; Pv, *Pvu*II; X, *Xho*I. pBRG700 contains IS*50* inserted into the Amp^r Tet^s plasmid pBR333, such that the 9 bp of pBR322 positions 31–39 are duplicated; pBR333 is missing pBR322 sequences 71 and 76 through 2352 (SASAKAWA, CARLE and BERG 1983; SASAKAWA *et al.* 1985). pBRG701 was derived from a pBR322::Tn*5* plasmid containing Tn*5* between *amp* and *rep* (HIRSCHEL and BERG 1982) by *in vitro* deletion of the segment containing *kan* and IS*50*L of Tn*5* and *amp* of pBR322, using *Sma*I to cleave the Tn*5* and *Eco*RI to cleave the pBR322 components, respectively (SASAKAWA, CARLE and BERG 1983).

Figure 2, the splicing sites were chosen such that the *tnp*$^+$ allele was close to the O end of the Tet^r transposon (0_1), and the *tnp*$^-$ allele was close to the O end of the Amp^r transposon (0_2). In the plasmids designated type II, the splicing at an alternative site reversed the positions of the *tnp*$^+$ and *tnp*$^-$ alleles in the Tet^r and Amp^r transposons. Because these pairs of plasmids were identical, except for the positions of the *tnp* alleles, the relative frequencies of Tet^r and of Amp^r transposition products should reflect how proximity of an O end to a functional *tnp*$^+$ gene affects transposition activity (Figure 2).

The heterodimeric plasmids were introduced into the λ lysogen DB1873, which is recombination deficient due to null alleles of the bacterial *recA* and prophage *red* genes. Phage λ development was induced in at least five separate subclones of each lysogen in each of two experiments. The relative yields of λTet^r and of λAmp^r transposition products in each lysate were determined by transduction of strain DB114. λTet^r phage were Amp^s and λAmp^r phage were Tet^s, as expected from previous findings that IS*50*-mediated transposition results in simple insertions, not cointegrates (BERG 1977, 1983). The median frequencies of Tet^r transductants relative to Amp^r transductants (Table 1) show that, with each of the five pairs of plasmids tested, the transposon whose O end was adjacent to the *tnp*$^+$ IS*50* element was more active than the complementary transposon whose O end was adjacent to the *tnp*$^-$ null allele.

To explain the inefficient transposition of segments bounded by pairs of I ends relative to those bounded by one O plus one I end, we (SASAKAWA and

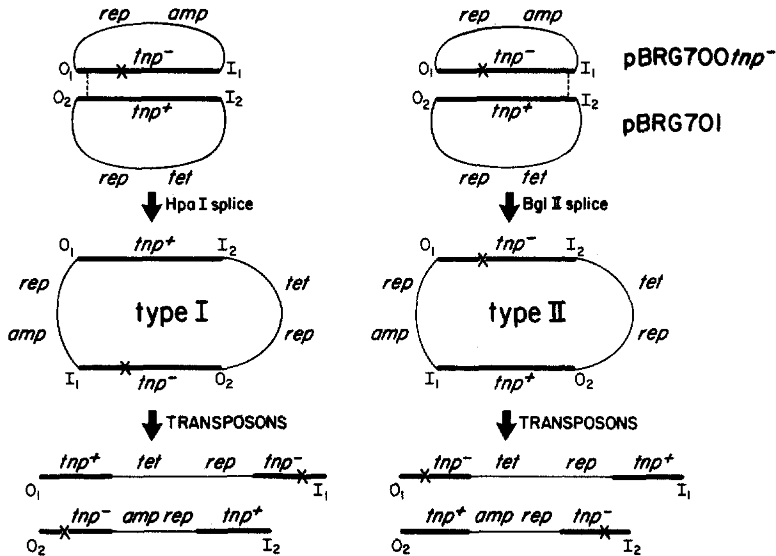

FIGURE 2.—Representative plasmids used to test effects of transposase gene position on transposition efficiency. Heterodimeric Amp^r Tet^r plasmids were generated *in vitro* by splicing, as indicated by the hypenated line, of pBRG700 (here diagramed with a linker insertion mutation "X" at the *Xho*I site) and pBRG701 at the *Hpa*I site near IS*50* O ends (left, "type I") and at the *Bgl*II site near IS*50* I ends (right, "type II"). Restriction endonuclease digestion (the appearance of fragments 3.3- and 6.1-kb long after digestion with *Hpa*I or *Xho*I) showed that, in each case, the monomeric components of the heterodimeric plasmids were joined in the orientation diagramed; joining in the opposite orientation would result in long, perfect palindromes, which have been shown to be deleterious or lethal in pBR322-based plasmids such as these (COLLINS, VOLCKAERT and NEVERS 1982; HAGAN and WARREN 1982). The Tet^r and Amp^r transposons and the positions of the tnp^+ and tnp^- alleles in them are also diagramed.

BERG 1982; BERG *et al.* 1982a) had proposed that transposase recognizes IS*50* ends in two steps—first an O end, and then either a second O end or the partially matched I end. Translation and transcription are coupled in bacteria. Hence, the *cis* action of transposase and the preference, albeit incomplete, for the most closely linked O ends seen here supports models (BERG *et al.* 1982a; MORISATO *et al.* 1984) in which the amino-terminus of a nascent transposase protein associates with DNA and in which the completed protein tracks along a DNA molecule in search of IS*50* ends. An association of nascent transposase with DNA would restrict diffusion of the *tnp* mRNA and, thus, keep additional proteins made from the same message near the gene in which they were encoded. Diffusion could be further restricted by the apparent binding of transposase to the cell membrane (ISBERG and SYVANEN 1985). Once transposase has associated with DNA nonspecifically, it may scan the DNA molecule until an O end sequence is found. The activity of any O end (Table 1) would then reflect its distance from the tnp^+ gene and also reflect rates at which transposase migrates and decays.

TABLE 1

Efficiency of transposition as a function of *tnp*$^+$ and *tnp*$^-$ allele positions

Position of *tnp*$^-$ allele	Ratio of Tet^r/Amp^r transposition products: Type I donor		Type II donor	
	Experiment I	Experiment II	Experiment I	Experiment II
189 (*Hpa*I)	1.6	1.5	0.50	0.47
487 (*Xho*I)	1.5	1.4	0.47	0.44
682 (*Pst*I)	1.7	1.5	0.79	0.73
1195 (*Hin*dIII)	1.3	1.4	0.54	0.50
1427 (*Pvu*II)	1.8	1.7	0.44	0.41

Type I and type II donors refer to the heterodimeric plasmids diagramed in Figure 2. They differ in the placement of *tnp*$^+$ and *tnp*$^-$ alleles relative to the IS*50* O ends used for the Tet^r and for the Amp^r transposons, designated 0_1 and 0_2, respectively.

The positions of the *Bam*HI linker (5′ CGGATCCG) insertions which created *tnp*$^-$ alleles are indicated in base pairs from the O end. These *tnp*$^-$ alleles were generated by linearizing plasmid DNAs with appropriate restriction endonucleases, followed by blunt-end ligation in the presence of the linkers. *Hpa*I and *Pvu*II digestions generate blunt ends; the 5′ extensions left by *Xho*I and *Hin*dIII were filled in with the Klenow fragment of DNA polymerase I, and the 3′ extension left by *Pst*I was removed with the 3′–5′ exonuclease activity of T4 DNA polymerase before ligation with the linkers. The *Xho*I, *Pst*I and *Pvu*II sites were mutated in pBRG700, and the *Hin*dIII site was mutated in pBRG701. *Pst*I and *Hin*dIII each cleave pBRG700 and pBRG701 plasmid DNAs at two places, and hence, mutation of these sites in IS*50* involved partial digestion of plasmid DNA and electrophoretic purification of the full-length linear product. Position 188 (*Hpa*I) was mutated separately in pBRG700 and in pBRG701. The positions of linkers at the expected sites were confirmed by restriction endonuclease digestion. Electrophoresis of end-labeled *Bam*HI fragments from plasmids mutant at *Xho*I and *Hin*dIII sites (as in BERG, SCHMANDT and LOWE 1983) showed that only one copy of the *Bam*HI linker was present; thus, the insertions at these sites did not change the reading frame.

The relative frequencies of transposition to phage λ were determined using derivatives of the *recA*$^-$ λ*red*$^-$ lysogen DB1873 carrying heterodimeric (Amp^r Tet^r) plasmids. Phage carrying the Amp^r and Tet^r markers derived by transposition were enumerated by transduction of the *hfl*-strain DB1891. Tet^r transductants were Amp^s, and vice versa, consistent with other findings that IS*50*-mediated transposition leads only to simple insertions, not cointegrates (the basis of our conclusion that IS*50*-mediated transposition is conservative) (BERG 1977; BERG 1983). The transposition frequencies listed in each experiment represent the median values from at least five independent lysates, each from a separate single-colony isolate. The median frequencies of Tet^r transposition products relative to free phage were measured as 2.3×10^{-7} and 2.1×10^{-7} per phage, using the *Hpa*I and *Pvu*II mutants of type I donors, respectively, and as 4.0×10^{-7} and 3.9×10^{-7} per phage, using the *Hpa*I and *Pvu*II mutants of type II donors, respectively.

We are grateful to C. M. BERG for critical readings of this manuscript. This work was supported by United States Public Health Service research grants AI14267 and AI18980 to D.E.B.

LITERATURE CITED

AUERSWALD, E., G. LUDWIG and H. SCHALLER, 1980 Structural analysis of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45:** 107–113.

BERG, D. E., 1977 Insertion and excision of transposable kanamycin resistance determinant Tn*5*. pp. 205–212. In: *DNA Insertion Elements, Plasmids and Episomes*, Edited by A. I. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

BERG, D. E., 1983 Structural requirement for IS*50*-mediated gene transposition. Proc. Natl. Acad. Sci. USA **79:** 792–796.

BERG, D. E. and C. M. BERG, 1983 The prokaryotic transposable element Tn*5*. Bio/technology **1:** 417–435.

BERG, D. E., J. DAVIES, B. ALLET and J.-D. ROCHAIX, 1975 Transposition of R factor genes to bacteriophage λ. Proc. Natl. Acad. Sci. USA **72:** 3628–3632.

BERG, D. E., L. JOHNSRUD, L. MCDIVITT, R. RAMBHADRAN and B. J. HIRSCHEL, 1982b The inverted repeats of Tn*5* are transposable elements. Proc. Natl. Acad. Sci. USA **79:** 2632–2635.

BERG, D. E., J. B. LOWE, C. SASAKAWA and L. MCDIVITT, 1982a The mechanism and control of Tn*5* transposition. pp. 5–28. In: *Fourteenth Stadler Genetics Symposium*, Edited by J. P. GUSTAFSON and G. REDEI. University of Missouri Press, Columbia, Missouri.

BERG, D. E., M. SCHMANDT and J. B. LOWE, 1983 Specificity of transposon Tn*5* insertion. Genetics **105:** 813–828.

COLLINS, J., G. VOLCKAERT and P. NEVERS, 1982 Precise and nearly precise excision of the symmetrical inverted repeats of Tn*5*: common features of *recA*-independent deletion events in *Escherichia coli.* Gene **19:** 139–146.

EGNER, C. and D. E. BERG, 1981 Excision of transposon Tn*5* dependent on the inverted repeats but not the transposase function of Tn*5*. Proc. Natl. Acad. Sci. USA **78:** 459–463.

HAGAN, C. E. and G. J. WARREN, 1982 Lethality of palindromic DNA and its use in the selection of recombinant plasmids. Gene **19:** 147–151.

HIRSCHEL, B. J. and D. E. BERG, 1982 A derivative of Tn*5* with direct terminal repeats can transpose. J. Mol. Biol. **155:** 105–120.

ISBERG, R. R. and M. SYVANEN, 1981 Replicon fusions promoted by the inverted repeats of Tn*5*: the right stem is an insertion sequence. J. Mol. Biol. **150:** 15–32.

ISBERG, R. R. and M. SYVANEN, 1985 Compartmentalization of the proteins encoded by IS*50*R. J. Biol. Chem. **260:** 3645–3651.

JOHNSON, R. C. and W. S. REZNIKOFF, 1981 Localization of the Tn*5* transposase promoter using the cycling reaction of RNA polymerase. Nucleic Acids Res. **9:** 1873–1883.

JOHNSON, R. C. and W. S. REZNIKOFF, 1983 DNA sequences at the ends of transposon Tn*5* required for transposition. Nature **340:** 280–282.

JOHNSON, R. C. and W. S. REZNIKOFF, 1984 Role of the IS*50*R protein in the promotion and control of Tn*5* transposition. J. Mol. Biol. **177:** 645–661.

LOWE, J. B. and D. E. BERG, 1983 A product of the transposase gene of Tn*5* inhibits Tn*5* transposition. Genetics **103:** 581–592.

MACHIDA, Y., C. MACHIDA, H. OHTSUBO and E. OHTSUBO, 1982 Factors determining frequency of plasmid cointegration mediated by insertion sequence IS*1*. Proc. Natl. Acad. Sci. USA **79:** 277–281.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular cloning.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MORISATO, D., J. WAY, H.-J. KIM and N. KLECKER, 1984 Tn*10* transposase acts preferentially on nearby transposon ends *in vivo.* Cell **32:** 799–807.

PATO, M. L. and C. REICH, 1984 Stoichiometric use of the transposase of bacteriophage Mu. Cell **36:** 197–202.

SASAKAWA, C. and D. E. BERG, 1982 IS*50* mediated inverse transposition: discrimination between the two ends of an IS element. J. Mol. Biol. **159:** 257–271.

SASAKAWA, C., G. F. CARLE and D. E. BERG, 1983 Sequences essential for transposition at the termini of IS*50*. Proc. Natl. Acad. Sci. USA **80:** 7293–7297.

SASAKAWA, C., S. H. PHADNIS, G. F. CARLE and D. E. BERG, 1985 Sequences essential for IS*50* transposition: the first base pair. J. Mol. Biol. **182:** 487–493.

WULFF, D. L. and M. ROSENBERG, 1983 Establishment of repressor synthesis. pp. 53–73. In: *Lambda II*, Edited by R. W. HENDRIX, J. W. ROBERTS, F. W. STAHL and R. A. WEISBERG. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Communicating editor: G. MOSIG