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Transposition of IS*50*$_L$ Activates Downstream Genes

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A transposition system constructed to detect the transposition of Tn*5* to a site upstream of the *lacZ* gene has revealed that transposition of IS*50*$_L$ can activate downstream genes. Expression is apparently mediated by the NPTII promoter. Transposase produced either by IS*50*$_R$ or by the suppressed IS*50*$_L$ catalyzed transposition of IS*50*$_L$.

Transposon Tn*5* is a composite transposable element that encodes resistance to kanamycin, streptomycin, and bleomycin (2, 3, 12) (Fig. 1A). Tn*5* contains two nearly identical insertion sequences (IS*50*) surrounding a 2.7-kilobase central region that encodes the antibiotic resistance genes (1, 6, 7, 11, 12, 15). Nucleotide sequence analysis (16) has verified that the two insertion sequences differ from each other at a single site. The base pair unique to IS*50*$_L$ not only results in an in-frame nonsense codon in the gene encoding the transposase (8) that is suppressible by the *supB* allele of *Escherichia coli*, but also forms part of the NPTII promoter sequence required for expression of the antibiotic resistance genes.

Building on the reported ability of Tn*5* to activate downstream genes as a consequence of its transposition (5), we designed an assay that might detect transposition events in individual colonies of *E. coli*. The crux of this transposition assay system is the promoter-probe vector, pRZ5202 (Fig. 1B; L. M. Munson, Ph.D. thesis, University of Wisconsin, Madison, 1983). This plasmid contains the *lacZ* gene from *E. coli*, which is fused at its 5′ end to the 3′ region of the *trpB* gene and thus lacks a promoter. The plasmid replicon was derived from pBR322 and also encodes resistance to ampicillin. We have used this vector to detect transposition of Tn*5* upstream of the *lacZ* gene, presuming that this would result in Lac$^+$ cells in the appropriate host. The strains used in this study (Table 1) contained intact *lacY* and *lacA* genes on an F factor, as well as the *recA56* allele. Isogenic pairs of strains harbored either no suppressor (*sup*$^+$) or the *supB* allele.

Strains RZ735 (*supB*) and RZ736 (*sup*$^+$) were infected with λ*bbnin*::Tn*5* or deletion derivatives (Table 1) and immediately plated on lactose-MacConkey agar containing 100 μg of ampicillin and 80 μg of kanamycin per ml. Within colonies of the kanamycin-resistant transductants, red (Lac$^+$) papillae appeared after 4 to 7 days of incubation at 30°C. Approximately 50% of the colonies arising by transduction with λ*bbnin*::Tn*5* contained at least one papilla after 7 days of incubation. The papillae were purified, and their plasmids were extracted and analyzed by restriction endonuclease digestion and agarose gel electrophoresis (14).

When wild-type Tn*5* (Fig. 1A) was the incoming transposon, restriction endonuclease analysis of the plasmids isolated from 29 Lac$^+$ clones showed that the papillae arose as a consequence of transposition of IS*50*, but not the entire Tn*5*, into pRZ5202. Assays of RNA polymerase-binding sites (10) in two of these clones indicated the presence of the NPTII promoter (Fig. 2), which showed that it was IS*50*$_L$ rather than IS*50*$_R$ which had been inserted upstream of the *lacZ* gene. Dideoxynucleotide sequencing (17) of appropriate portions of these plasmids confirmed this identification (data not shown). Papillation occurred at a slightly higher frequency in the *supB* strain than in the *sup*$^+$ strain (data not shown). These findings agree with those of Berg et al. (4), which indicated that IS*50*$_L$ can transpose independently of IS*50*$_R$ (provided that transposase is present) and that Tn*5* transposase activity is diffusible, catalyzing the autonomous transposition of a nearby insertion element. Our results show also that transposition of IS*50*$_L$ in the correct orientation strongly activates the *lacZ* gene.

The characterized insertions that generated the Lac$^+$ phenotype upon transposition of Tn*5* resulted from transposition of IS*50*$_L$ into the smaller *Eco*RI-*Cla*I fragment of pRZ5202 such that the NPTII promoter was proximal to the *lacZ* gene (cf. pRZ602 in Fig. 3; Fig. 4). This finding suggested that transposition of IS*50*$_L$ in the opposite orientation was incapable of generating a Lac$^+$ clone, presumably because there was no outward-directed promoter of suffi-

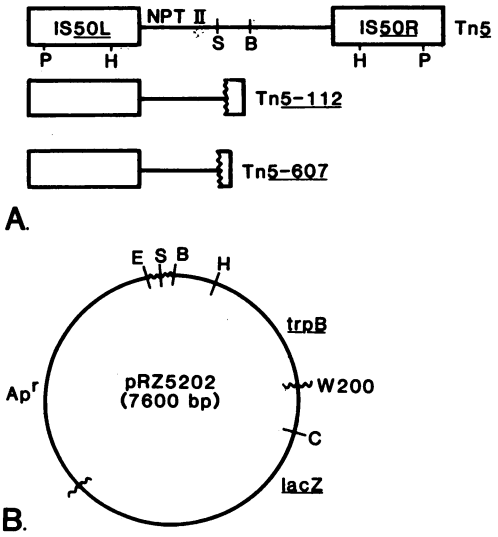

FIG. 1. (A) Transposon Tn*5* and deletion derivatives used in this study. (B) Diagram of the promoter probe vector, pRZ5202 (Munson, Ph.D. thesis). Relevant restriction sites are indicated and abbreviated as follows: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Hpa*I; S, *Sma*I. bp, Base pairs.

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TABLE 1. Bacterial strains, phage strains, and plasmids used in this study

Strain or plasmid	Markers[a]	Source
E. coli strains		
RZ704	Δ(*pro-lac*) *ara thi rpsL supB recA56 srl*	This laboratory
RZ723	RZ704 [F′Δ(*lacO-lacZ*)*S20*]	This study
RZ735	RZ723(pRZ5202)	This study
RZ736	RZ735 *sup*$^+$	This study
Lambda strains		
λ*bbnin*	*c*I857 *b515 b519 nin5 S*am7	R. Young
λ*bbnin*::Tn*5*		Transposition from pRZ102 onto λ*bbnin*
λ*bbnin*::Tn*5-112*		Transposition from pRZ112 onto λ*bbnin*
λ*bbnin*::Tn*5-607*		Transposition from pRZ607 onto λ*bbnin*
λ*int*::Tn*5*	*c*I857 *b221 O*am29 *P*am80 *rex*::Tn*5*	N. Kleckner
Plasmids		
pRZ102	ColE1::Tn*5* (Km^r)	Jorgensen et al. (11)
pRZ112	ColE1::Tn*5-112* (Km^r)	Jorgensen et al. (11)
pRZ607	ColE1::Tn*5-607* (Km^r)	*Bal*31-generated deletion of pRZ102
pRZ5202	φ(*trp-lac*)W200 (Ap^r)	Munson, Ph.D. thesis
pRZ600	pRZ5202::Tn*5* (Km^r Ap^r)	Transposition from λ::Tn*5*
pRZ601	pRZ5202::IS*50*$_R$ (Ap^r)	Deletion of *Bam*HI fragment from pRZ600
pRZ602	pRZ5202::IS*50*$_L$ (Lac$^+$ Ap^r)	Transposition from λ::Tn*5*
pRZ608	pRZ5202::Tn*5-607* (Km^r Lac$^+$ Ap^r)	Transposition from λ::Tn*5-607*
pRZ609	pRZ5202::IS*50*$_L$ (Km^r Ap^r)	Insertion of *Eco*RI-*Bam*HI fragment from pRZ102 into *Eco*RI-*Bam*HI-digested pRZ5202
pRZ610	pRZ5202::IS*50*$_L$ (Km^r Ap^r)	Replacement of *Bam*HI-*Hin*dIII fragment of pRZ601 with *Bam*HI-*Hin*dIII fragment of pRZ102
pRZ611	pRZ5202::IS*50*$_L$ (Lac$^+$ Ap^r)	Transposition from λ::Tn*5-607*

[a] Abbreviations: Km^r, kanamycin resistance; Ap^r, ampicillin resistance; Lac$^+$, growth on lactose.

cient strength to express *lacZ*. Our assumption was confirmed by the analysis of β-galactosidase activities generated by a variety of transposition products and artificially constructed derivatives of pRZ5202 containing various portions of Tn*5*; high levels of β-galactosidase were produced only from those plasmids in which the NPTII promoter was adjacent to the *lacZ* gene (Fig. 3). Berg et al. (5) suggested the existence of an outward-directed promoter on IS*50*$_R$. If such a control region does exist, it promotes only weak expression of β-galactosidase (cf. pRZ600 in Fig. 3). We found no evidence for strong activation of *lacZ* by IS*50*$_R$.

In a separate experiment, we verified that the insertion of IS*50* upstream of *lacZ* in pRZ5202 occurred independently of the presence of bacteriophage λ. Strain RZ723 was infected with λ*int*::Tn*5*, which could neither replicate nor integrate. A kanamycin-resistant transductant was purified, and the absence of λ was confirmed by demonstrating both heat resistance and a lack of superinfection immunity. This isolate was transformed with pRZ5202 and plated on lactose-MacConkey agar containing ampicillin and kanamycin. Lac$^+$ papillae appeared at a frequency similar to that found in the standard assay described above; all papillae that were isolated and characterized showed the insertion of IS*50* in

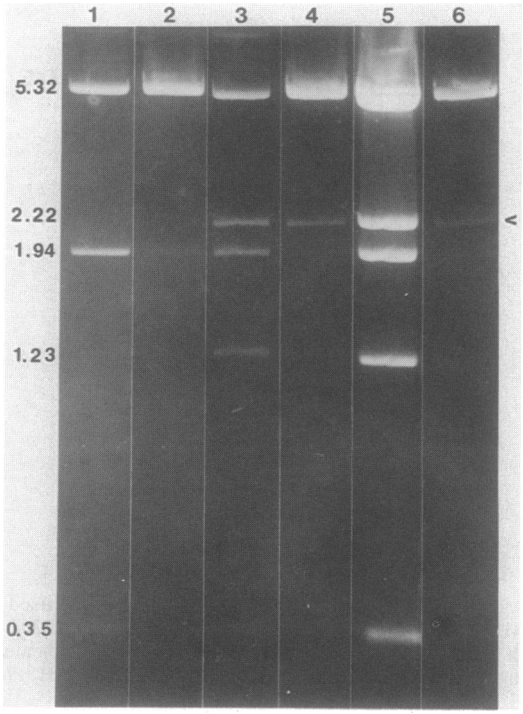

FIG. 2. RNA polymerase-binding assays. Purified plasmids were digested with *Eco*RI, *Hin*dIII, and *Cla*I. DNA fragments to which RNA polymerase had bound (10) were resolved by electrophoresis on a 1.5% agarose gel for comparison with untreated DNA fragments. The gel was stained with ethidium bromide and photographed by UV irradiation. Lanes: 1 and 2, pRZ5202; 3 and 4, pRZ602; 5 and 6, pRZ611; 1, 3, and 5, untreated DNA fragments; 2, 4, and 6, DNA fragments treated with RNA polymerase. Fragment sizes are indicated in kilobase pairs. Transposition of IS*50*$_L$ into the smaller *Eco*RI-*Cla*I fragment of pRZ5202 generated an RNA polymerase-binding *Hin*dIII-*Cla*I fragment of ca. 2,220 base pairs, indicated by the arrowhead. Both pRZ602 and pRZ611 were generated by transposition of IS*50*$_L$ into a dimer of pRZ5202 and therefore retained one copy of the 1.94-kilobase-pair *Hin*dIII-*Cla*I fragment. Control experiments verified that RNA polymerase did not bind to the inside end of IS*50*$_R$ (data not shown; 15). Therefore, transposition of IS*50*$_R$ upstream of the *lacZ* gene would generate a *Hin*dIII-*Cla*I fragment that would not interact with RNA polymerase.

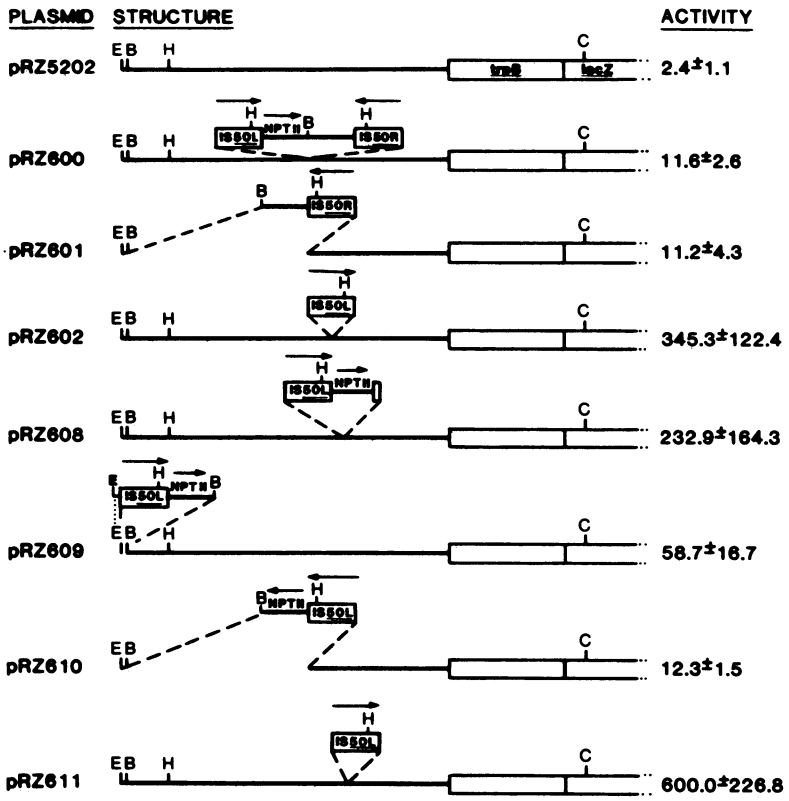

FIG. 3. Plasmid structures and corresponding β-galactosidase activities (measured by the procedure of Miller [13]). Only the portions of the plasmids between the *Eco*RI site and the 3′ region of *lacZ* are shown. For each plasmid, the number of trials exceeded seven. Abbreviations: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII. Arrows indicate the directions of transcription of genes within the insertion sequences ($IS50_L$ and $IS50_R$) and the neomycin phosphotransferase (NPTII) gene. Activity is expressed as nanomoles of *o*-nitrophenol produced per minute per A_{600} unit (13).

the smaller *Eco*RI-*Cla*I fragment of pRZ5202 in the expected orientation.

Two deletion mutants of Tn*5* also gave rise to Lac$^+$ papillae upon transposition into pRZ5202. Transposition of Tn*5-112* (Fig. 1A) generated Lac$^+$ papillae at a high frequency in a *supB* host but not in a *sup*$^+$ host. In the suppressor strain, approximately 25% of the Lac$^+$ papillae arose as the result of transposition of $IS50_L$ alone, whereas the remainder arose as a consequence of transposition of the entire Tn*5-112*. In the latter instance, plasmids isolated from the Lac$^+$ clones conferred both ampicillin and kanamycin resistance. The orientation of the transposon in these cases was such that the truncated $IS50_R$ was closer to the *lacZ* gene. Similar results were obtained with a second deleted derivative, Tn*5-607* (Fig. 1A). The plasmids isolated from the rare Lac$^+$ clones arising in the *sup*$^+$ host were complex, and the origin of the Lac$^+$ phenotype was inexplicable. Products originating by transposition of Tn*5-607* into pRZ5202 showed high β-galactosidase activities (cf. pRZ608 and pRZ611 in Fig. 3). We infer from these results that the NPTII promoter can direct transcription through the outermost end of $IS50_R$ in these deleted derivatives but not in the intact Tn*5*.

For the Tn*5* derivatives tested, the frequency of papillation was proportional to the frequency of transposition of the entire transposon when measured by either transduction assay (15) or mating assay (9) (data not shown). Furthermore, transposition of the two deletion derivatives, both of which lacked intact $IS50_R$, was dependent on the *supB* allele. This result indicated that we were detecting transposition events which used the Tn*5* transposase encoded by the suppressed $IS50_L$.

The transposition assay system described here has permitted the detection of the independent transposition of $IS50_L$. An important evolutionary consequence of transposition of $IS50_L$ is likely to be the activation of silent genes.

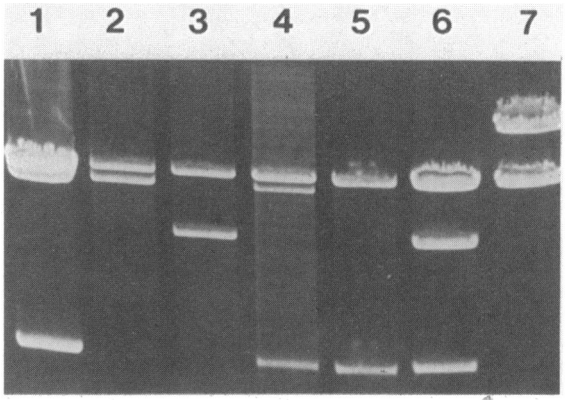

FIG. 4. Analysis of plasmids generated by transposition of $IS50_L$ or Tn*5* from λ::Tn*5* to pRZ5202. Plasmids were digested with *Eco*RI and *Cla*I and resolved by electrophoresis in a 1% agarose gel. Lanes: 1, pRZ608; 2 through 4, other products of transposition from λ::Tn*5-607*; 5, pRZ5202; 6, pRZ602; 7, pRZ600. Of these examples, only the colonies containing the plasmids shown in lanes 1 through 4 and lane 6 were Lac$^+$. The plasmids in lanes 1, 4, and 6 were generated by transposition into a dimer of pRZ5202.

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