

# The *insE* Open Reading Frame of *IS1* Is Not Required for Formation of Cointegrates

ELAINE T. FREUND† AND MIRIAM M. SUSSKIND\*

Hedco Molecular Biology Laboratories, University of Southern California,  
 Los Angeles, California 90089-1340

Received 18 April 1995/Accepted 26 January 1996

**The role of the *insE* open reading frame in transposition of *IS1* was reexamined by using an *insE* nonsense mutation that does not alter the amino acid sequence of InsA inhibitor or InsAB transposase. The mutant was active in all strains tested, showing that *insE* is not essential for formation of cointegrates.**

Two open reading frames (ORFs) of the transposable element *IS1*, *insA* and *insB*, are required for transposition (10, 12). Faithful translation of *IS1* transcripts produces InsA, a 91-residue protein with a putative helix-turn-helix DNA-binding motif near the C terminus. InsA binds specifically to the ends of *IS1* (22) and inhibits transposition. Evidence also suggests that InsA represses *IS1* transcription (11, 23). The transposase of *IS1*, InsAB, is produced by a programmed translational frameshift from the *insA* (0) frame to the *insB* (−1) frame. Fewer than 1% of the ribosomes translating the A frame shift to the B frame, so that much more InsA than InsAB is made. The ratio of InsA to InsAB appears to be important in the regulation of transposition, possibly because the two proteins compete for binding to the ends of *IS1* (5, 16, 17, 23).

There are eight ORFs (*insA* through *insG*) of *IS1* that could potentially encode proteins of 50 or more amino acids (Fig. 1). In order to determine which ORFs were necessary for cointegrate formation, Jakowec et al. (10) created amber mutations in each ORF, leaving overlapping reading frames unaffected when possible. The only ORFs required for cointegrate formation in *Escherichia coli* were *insA* and *insB*. Since *E. coli* K-12 contains several chromosomal copies of wild-type *IS1*, which can potentially provide transposition functions in *trans* (1, 4), the *IS1* mutants were also tested in *Salmonella typhimurium* LT2, which has no copies of *IS1* (13). As expected, cointegrate formation in *S. typhimurium* required *insA* and *insB*. In addition, an *insE* mutant showed a significant reduction in the ability to form cointegrates. Jakowec et al. (10) suggested that *insE* may encode an accessory protein that is provided in *trans* by chromosomal copies of *IS1* in *E. coli*.

The original *insE* amber mutation (called *insE-am328* here) is located in a region that is now known to program the frameshift during synthesis of InsAB transposase (5, 16, 17). Therefore, the *insE* mutation used by Jakowec et al. (10) does not simply affect the *insE* ORF; it also changes the amino acid sequence of InsAB (Table 1). To reassess the role of *insE* in transposition, a different *insE-am* mutation (*insE-am426*) that is more than 100 bp away from the frameshift site and does not change the amino acid sequence of InsAB was used. Various *IS1* plasmids were tested for transposition by a mating-out

assay. We find no evidence that the *insE* ORF functions in *IS1* transposition.

***IS1* plasmids.** pZIS1 (Fig. 2) and its derivatives carrying mutations in *IS1* are described in Table 2. DNA sequencing confirmed that the original *insE* mutant plasmid, pMJ13 (10), carries *insE-am328* and no other mutation in the 200-bp *MscI-MluI* region. Fragment exchanges were carried out to construct a derivative of pZIS1 that has only the *MscI-MluI* region from pMJ13, to ensure that the resulting plasmid (pMS1409) has only the *insE-am328* mutation. Plasmid pMJ73 carries another *insE* amber mutation, *insE-am426*, which was constructed by M. Jakowec but was not previously characterized. Plasmid pMJ5 carries *insA-am179*, an amber mutation that prevents synthesis of InsA and InsAB (10).

**pOX38Km *finP* conjugal plasmid.** The products of the *finO* and *finP* genes inhibit transfer of F and F-like conjugal plasmids (19, 20). pOX38Km, a *finO finP*<sup>+</sup> derivative of F, transfers constitutively in *E. coli*. *S. typhimurium* LT2, however, has an endogenous plasmid that provides *FinO* in *trans* (7), reducing transfer of pOX38Km. In order to carry out mating-out assays with *S. typhimurium*, we made a derivative of pOX38Km carrying a *finP* mutation, which alleviates fertility inhibition. This was done in two steps. First, a Mud-P22 insertion (21) was crossed onto F' *lac finP*, and then this strain was used to cross the *finP* mutation onto pOX38Km without selection.

*S. typhimurium* PY13761 (21) (Table 3) carries F' *ts114 lac* with a Tn10 insertion and a Mud-P22 insertion, which confer resistance to tetracycline and chloramphenicol, respectively.

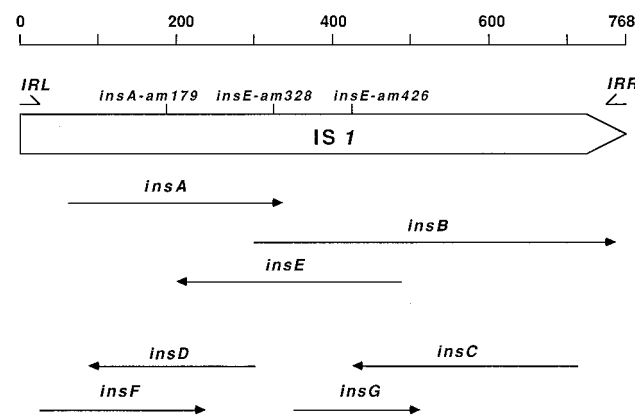


FIG. 1. Open reading frames of *IS1*. IRL and IRR, inverted repeat left and right, respectively. Arrows indicate the directions of the ORFs.

\* Corresponding author. Mailing address: University of Southern California, Program in Molecular Biology, Los Angeles, CA 90089-1340. Phone: (213) 740-5553. Fax: (213) 740-8631.

† Present address: Pomona College, Program in Molecular Biology, Claremont, CA 91711.

TABLE 1. Effects of mutations on IS1 ORFs

IS1 mutation	Effect on IS1 ORF <sup>a</sup> :		
	A	AB	E
<i>insA-am179</i>	Gln-40→amber	Gln-40→amber	None
<i>insE-am328</i>	Silent (Arg-91)	Val-92→Leu	Tyr-47→amber
<i>insE-am426</i>	None	Silent (Leu-124)	Gln-15→amber

<sup>a</sup> The effects of each mutation at the amino acid level are listed. Nucleotide changes are given in Table 2. AB refers to the sequence of InsAB protein, which is synthesized by a programmed shift from the A frame to the B frame.

Because the Mud-P22 prophage cannot excise, mitomycin induction of PY13761 at 30°C leads to in situ replication and packaging of F' *lac* Tn10 Mud-P22 DNA. The resulting stock of particles was saturated with P22 tail protein (since Mud-P22 is missing the P22 tail gene). Various dilutions of this stock were mixed with *S. typhimurium* MS3152, which carries F' *lac finP* (7). Recombinants that acquired Mud-P22 were selected by plating on LB-LS (Luria broth containing a lower concentration of salt) (18) plates containing chloramphenicol (20 µg/ml) at 30°C. These recombinants carried F' *ts<sup>+</sup> lac finP* Mud-P22, since they were stably Lac<sup>+</sup> at 40°C, immune to P22 ant (i.e., carried the P22 *immC* region), Tet<sup>s</sup> (i.e., did not carry the Tn10 insertion in F), and FinP<sup>-</sup> (i.e., sensitive to phage M13). One of these recombinants was induced with mitomycin, and the resulting stock of particles was saturated with P22 tail protein. Particles were adsorbed to *S. typhimurium* MS3371 carrying pOX38Km, and after outgrowth in LB-LS, the cells were plated on MacConkey agar (Difco) supplemented with 1% (wt/vol) lactose. White (Lac<sup>-</sup>) colonies were tested for sensitivity to M13. One *finP* recombinant was found among 727 colonies tested. The pOX38Km *finP* plasmid in this strain confers resistance to kanamycin (i.e., retains the *kan* gene of pOX38Km), does not confer P22 immunity (i.e., does not carry Mud-P22), allows efficient plaque formation by M13, and transfers at high efficiency to appropriate recipients (data not shown).

**Measurement of frequency of cointegrates.** The Tet<sup>r</sup> plasmids containing wild-type or mutant versions of IS1 were introduced by transformation into isogenic *E. coli* *recA<sup>+</sup>* and *recA* strains (MC4100 and GE999) and into isogenic *S. typhimurium* *recA<sup>+</sup>* and *recA* strains (MS1868 and TP134). The Kan<sup>r</sup> plasmid pOX38Km *finP* was then transferred into each strain by conjugation. IS1 transposition proficiency was measured in the mating-out assay, in which the target Kan<sup>r</sup> plasmid pOX38Km *finP* was transferred by conjugation to an *E. coli* recipient resistant to nalidixic acid and rifampicin. Recipients can acquire Tet<sup>r</sup> as well as Kan<sup>r</sup> if the Tet<sup>r</sup> IS1 plasmid under-

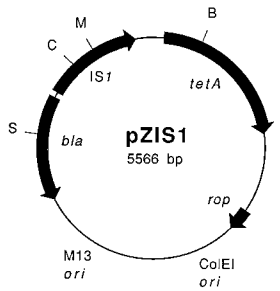


FIG. 2. Structure of pZIS1. pZIS1 is a pBR322-based plasmid carrying IS1 clockwise between the *bla* and *tetA* genes. It carries both the ColEI and M13 origins of replication. B, *Bam*HI; C, *Msc*I; M, *Mlu*I; S, *Sca*I.

TABLE 2. Plasmids

Plasmid	Description	Reference or source
pZIS1	Derivative of pZ152 (21a) carrying IS1 inserted between <i>bla</i> and <i>tetA</i>	14
pMJ5	Derivative of pZIS1 carrying <i>insA-am179</i> (C→T at IS1 bp 179) and A→G at IS1 bp 175	10
pMJ13	Derivative of pZIS1 carrying <i>insE-am328</i> (G→C at IS1 bp 328)	10
pMJ73	Derivative of pZIS1 carrying <i>insE-am426</i> (G→A at IS1 bp 426)	M. Jakowec
pMS1400	IS1 <i>insE-am328 Bam</i> HI- <i>Msc</i> I fragment from pMJ13 and the <i>Bam</i> HI- <i>Msc</i> I backbone of pZIS1	This work
pMS1409	IS1 <i>insE-am328 Mlu</i> I- <i>Sca</i> I fragment from pMS1400 and the <i>Mlu</i> I- <i>Sca</i> I backbone of pZIS1	This work
pOX38Km	F-based conjugative plasmid carrying the <i>kan</i> gene from Tn5	3
pOX38Km <i>finP</i>	<i>finP</i> derivative of pOX38Km	This work

goes cointegration with the target Kan<sup>r</sup> plasmid in the donor. The assay measures the frequency of Tet<sup>r</sup> among the Kan<sup>r</sup> transconjugants.

A modification of the mating-out assay described by Chandler and Galas (3) was used. Fresh overnight cultures grown in LB-HS (Luria broth containing a higher concentration of salt) (15) plus antibiotics were diluted 100-fold in LB-HS without antibiotics. Cultures were grown at 37°C with agitation to a density of about 2 × 10<sup>8</sup> cells per ml. While the recipient cells continued to be shaken, the donors were incubated for 30 min without agitation to enhance pilus formation. The donor and recipient cultures were then mixed together in equal volumes and shaken gently for 90 min. Mating was disrupted by agitation and chilling on ice or at 4°C. Suitable dilutions of the mating mixture were plated onto selective media containing appropriate combinations of antibiotics (kanamycin, 10 µg/ml; nalidixic acid, 17 µg/ml; rifampicin, 50 µg/ml; and tetracycline, 10 µg/ml).

TABLE 3. Bacterial strains

Strain	Genotype	Reference or source
<i>Escherichia coli</i>		
GE999	MC4100 $\Delta$ <i>recA</i> 1398	G. Weinstock
HB101	<i>hsdS20</i> (r <sup>-</sup> m <sup>-</sup> ) <i>recA13</i> <i>ara14</i> <i>proA2</i> <i>lacY1</i> <i>galK2</i> <i>rpsL20</i> <i>xyl-5</i> <i>mtl-1</i> <i>supE44</i>	15
MC4100	F <sup>-</sup> <i>araD139</i> $\Delta$ ( <i>argF-lac</i> )U169 <i>rpsL150</i> <i>relA1</i> <i>flbB5301</i> <i>deoC1</i> <i>ptsF25</i> <i>rbsR</i>	G. Weinstock
MS4086	HB101 <i>nal</i> <i>rif</i>	2
<i>Salmonella typhimurium</i> LT2		
MS1868	<i>leuA-am414 hsdSA</i> (r <sup>-</sup> m <sup>+</sup> )	9
MS3152	MS1868/F' <i>lac finP</i>	This work
MS3371	MS1868 <i>str</i>	8
MS3687	MS3371/pOX38Km <i>finP</i>	This work
PY13761	<i>leuA-am414 hsdSA</i> (r <sup>-</sup> m <sup>+</sup> )/F' <i>ts114 lac zcf-20::Tn10 zcf-3557::Mud-Q</i>	21
TP134	<i>leuA-am414 hsdSA recA</i>	6

TABLE 4. Frequencies of cointegrates<sup>a</sup>

Host and plasmid	IS1	Frequency of cointegrates in <sup>b</sup> :	
		<i>recA</i> <sup>+</sup> strains	<i>recA</i> strains
<i>E. coli</i>			
pBR322	None	$<(1.9 \pm 0.5) \times 10^{-8}$ [4]	$<(4.1 \pm 0.6) \times 10^{-8}$ [4]
pZIS1	Wild type	$(3.2 \pm 1.2) \times 10^{-6}$ [4]	$(1.1 \pm 0.1) \times 10^{-6}$ [4]
pMJ5	<i>insA-am179</i>	$(6.8 \pm 2.3) \times 10^{-8}$ [4]	$(9.0 \pm 1.6) \times 10^{-8}$ [4]
pMS1409	<i>insE-am328</i>	$(1.0 \pm 0.3) \times 10^{-6}$ [7]	$(2.9 \pm 1.0) \times 10^{-7}$ [6]
pMJ73	<i>insE-am426</i>	$(7.8 \pm 1.8) \times 10^{-7}$ [4]	$(2.7 \pm 0.5) \times 10^{-6}$ [4]
<i>S. typhimurium</i>			
pBR322	None	$(5.6 \pm 2.0) \times 10^{-8}$ [5]	$<(3.2 \pm 1.4) \times 10^{-8}$ [6]
pZIS1	Wild type	$(5.7 \pm 0.7) \times 10^{-6}$ [5]	$(4.2 \pm 0.6) \times 10^{-7}$ [6] <sup>c</sup>
pMJ5	<i>insA-am179</i>	$(6.2 \pm 1.8) \times 10^{-8}$ [5]	$(8.4 \pm 3.9) \times 10^{-8}$ [6]
pMS1409	<i>insE-am328</i>	$(3.3 \pm 0.4) \times 10^{-7}$ [5]	$<(1.2 \pm 0.3) \times 10^{-7}$ [6]
pMJ73	<i>insE-am426</i>	$(4.9 \pm 0.7) \times 10^{-6}$ [5]	$(7.7 \pm 1.3) \times 10^{-7}$ [6]

<sup>a</sup> Donors were derivatives of MC4100, GE999, MS1868, and TP134 carrying pOX38Km *finP* (Kan<sup>r</sup>) and the indicated Tet<sup>r</sup> plasmids. The recipient was *E. coli* HB101 *nal* rif<sup>r</sup> (MS4086).

<sup>b</sup> The frequency of cointegrates is the number of Tet<sup>r</sup> Kan<sup>r</sup> Nal<sup>r</sup> Rif<sup>r</sup> transconjugants (which occur by transfer of a cointegrate between pOX38Km *finP* and the IS1 plasmid) divided by the number of Kan<sup>r</sup> Nal<sup>r</sup> Rif<sup>r</sup> transconjugants (which occur by transfer of pOX38Km *finP*). The mean and standard error are given, and the number of measurements is in brackets.

<sup>c</sup> The *recA* mutation in *S. typhimurium* reduces the frequency of cointegrates with wild-type IS1 more than 10-fold. The reason for this is unclear. Consequently, the differences between active and inactive IS1 elements are diminished.

**Effect of *insE* mutations on the frequency of cointegrates.**

Table 4 shows that in the *recA*<sup>+</sup> strains, the *insA-am179* mutation reduced the frequency of cointegrates 50- to 100-fold; this shows that cointegrates are mediated by IS1. The *insE-am328* mutation reduced the frequency of cointegrates more than 10-fold in *S. typhimurium* but had little effect in *E. coli*, in agreement with the results of Jakowec et al. (10), who reported that the activity of this mutant was normal in *E. coli* *recA*<sup>+</sup> *thyA*<sup>+</sup> but was reduced 70-fold in *S. typhimurium* *recA*<sup>+</sup> *thyA*. In contrast, the *insE-am426* mutant was not severely defective in either *E. coli* or *S. typhimurium*.

Results obtained with the *E. coli* and *S. typhimurium* *recA* donors were near the limits of detection of the assay; therefore, differences between active and inactive IS1 elements were diminished. As expected, the *insA-am179* mutation reduced the frequency of cointegrates in both *E. coli* and *S. typhimurium*, demonstrating that most cointegrates are IS1 mediated. In both bacteria, the *insE-am328* mutant was not fully active, whereas the *insE-am426* mutant was fully active in cointegrate formation.

Jakowec et al. (10) suggested that the *insE* ORF plays a role in IS1 transposition. Their observation that the *insE-am328* mutation severely diminished transposition activity in *S. typhimurium* LT2, but not in *E. coli*, implied that the chromosomal copies of IS1 in the *E. coli* genome were complementing the *insE* mutant in *trans*. However, the original *insE-am328* mutation is now known to be located in the frameshift signal region and to cause an amino acid change in InsAB. The previously uncharacterized *insE-am426* mutant has a change that does not alter the amino acid sequence of InsA or InsAB. This mutant was transpositionally active in all strains tested. This shows that the *insE* ORF is not important in transposition. The results with *insE-am328* can be attributed to the amino acid change in InsAB, to effects on translational frameshifting, and/or to effects on transcription termination. Because the *am328* mutation lies in a complicated regulatory region, it is not surprising that the severity of the mutant phenotype differs in *S. typhimurium* and *E. coli*.

This work was supported by National Institutes of Health grants GM36811 and AI19036-13.

We thank Richard Deonier for helpful suggestions and discussions and George Weinstock for providing strains.

**REFERENCES**

- Birkenbihl, R. P., and W. Vielmetter. 1989. Complete maps of IS1, IS2, IS3, IS4, IS5, IS30, and IS50 location in *E. coli* K12. *Mol. Gen. Genet.* **220**: 147-153.
- Chakrabarti, R. 1989. Ph.D. thesis. University of Southern California, Los Angeles.
- Chandler, M., and D. J. Galas. 1983. Cointegrate formation mediated by Tn9. II. Activity of IS1 is modulated by external DNA sequences. *J. Mol. Biol.* **170**:61-91.
- Deonier, R. C. 1987. Locations of native insertion sequence elements, p. 982-989. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
- Escoubas, J. M., M. F. Prere, O. Fayet, I. Salvignol, D. Galas, D. Zerbib, and M. Chandler. 1991. Translational control of transposition activity of the bacterial insertion sequence IS1. *EMBO J.* **10**:705-712.
- Fenton, S. C., and A. R. Poteete. 1984. Genetic analysis of the *erf* region of the bacteriophage P22 chromosome. *Virology* **134**:148-160.
- Finnegan, D. J., and N. S. Willetts. 1971. Two classes of *Flac* mutants insensitive to transfer inhibition by an F-like R factor. *Mol. Gen. Genet.* **111**: 256-264.
- Franko, M. 1993. Ph.D. thesis. University of Southern California, Los Angeles.
- Graña, D., T. Gardella, and M. M. Susskind. 1988. The effects of mutations in the *ant* promoter of phage P22 depend on context. *Genetics* **120**:319-327.
- Jakowec, M., P. Prentki, M. Chandler, and D. J. Galas. 1988. Mutational analysis of the open reading frames in the transposable element IS1. *Genetics* **120**:47-55.
- Machida, C., and Y. Machida. 1989. Regulation of IS1 transposition by the *insA* gene product. *J. Mol. Biol.* **208**:567-574.
- Machida, Y., C. Machida, and E. Ohtsubo. 1984. Insertion element IS1 encodes two structural genes required for its transposition. *J. Mol. Biol.* **177**: 229-245.
- Nyman, K., K. Nakamura, H. Ohtsubo, and E. Ohtsubo. 1981. Distribution of the insertion sequence IS1 in gram-negative bacteria. *Nature (London)* **289**:609-612.
- Prentki, P., B. Teter, M. Chandler, and D. J. Galas. 1986. Functional promoters created by the insertion of the transposable element IS1. *J. Mol. Biol.* **191**:383-393.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Sekine, Y., and E. Ohtsubo. 1989. Frameshifting is required for production of the transposase encoded by insertion sequence I. *Proc. Natl. Acad. Sci. USA* **86**:4609-4613.
- Sekine, Y., and E. Ohtsubo. 1992. DNA sequences required for translational

- frameshifting in production of transposase encoded by *IS1*. *Mol. Gen. Genet.* **235**:325–332.
18. **Susskind, M. M., A. Wright, and D. Botstein.** 1971. Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. II. Genetic evidence for two exclusion systems. *Virology* **45**:638–652.
  19. **Willets, N., and R. Skurray.** 1987. F Factor and conjugation, p. 1110–1133. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
  20. **Yoshioka, Y., H. Ohtsubo, and E. Ohtsubo.** 1987. Repressor gene *finO* in plasmids R100 and F: constitutive transfer of plasmid F is caused by insertion of *IS3* into F *finO*. *J. Bacteriol.* **169**:619–623.
  21. **Youderian, P., P. Sugiono, K. L. Brewer, N. P. Higgins, and T. Elliot.** 1988. Packaging specific segments of the *Salmonella* chromosome with locked-in Mud-P22 prophages. *Genetics* **118**:581–592.
  - 21a. **Zagursky, R. J., and M. L. Berman.** 1984. Cloning vectors that yield high levels of single-stranded DNA for rapid DNA sequencing. *Gene* **27**:183–191.
  22. **Zerbib, D., M. Jakowec, P. Prentki, D. J. Galas, and M. Chandler.** 1987. Expression of proteins essential for *IS1* transposition: specific binding of *InsA* to the ends of *IS1*. *EMBO J.* **6**:3163–3169.
  23. **Zerbib, D., P. Polard, J. M. Escoubas, D. Galas, and M. Chandler.** 1990. The regulatory role of the *IS1*-encoded *InsA* protein in transposition. *Mol. Microbiol.* **4**:471–477.