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

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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. InsB 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 Mos1-MluI region. Fragment exchanges were carried out to construct a derivative of pZIS1 that has only the Mos1-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 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 Mu-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 Mup-P22 insertion, which confer resistance to tetracycline and chloramphenicol, respectively.
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' lac finP Mud-P22, since they were stably Lac+ at 40°C, immune to P22 anti (i.e., carried the P22 immC region), Tet+ (i.e., did not carry the Tn10 insertion in F), and FinF' (i.e., sensitive to phase 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 MS3731 carrying pOX38Km, and after outgrowth in LB-LS, the cells were plated on MacConkey agar (Difco) supplemented with 1% (wt/vol) lactose. White (Lac−) 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+ plasmids containing wild-type or mutant versions of IS1 were introduced by transformation into isogenic E. coli recA− and recA strains (MC4100 and GE999) and into isogenic S. typhimurium recA− and recA strains (MS1868 and TP134). The Kan' 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' plasmid pOX38Km finP was transferred by conjugation to an E. coli recipient resistant to nalidixic acid and rifampicin. Recipients can acquire Tet' as well as Kan' if the Tet' IS1 plasmid undergoes cointegration with the target Kan' plasmid in the donor. The assay measures the frequency of Tet' among the Kan' 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 × 108 cells/ml. While the recipient cells continued to be shaken, the donors were incubated for 30 min without agitation to enhance plus 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 on 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 1. Effects of mutations on IS1 ORFs

| IS1 mutation | Effect on IS1 ORFs: |
|--------------|-------------------|
|              | A                 | AB               | E             |
| insA-am4179  | Gln-40→amber     | Gln-40→amber     | None          |
| insE-am328   | Silent (Arg-91)   | Val-92→Leu      | Tyr-47→amber  |
| insE-am426   | None              | Silent (Leu-124) | Gln-15→amber  |

* 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.

*FIG. 2. Structure of pZIS1. pZIS1 is a pBR322-based plasmid carrying IS1 clockwise between the bla and tetA genes. It carries both the ColE1 and M13 origins of replication. B, BamHI; C, MscI; M, MluI; S, ScaI.

TABLE 2. Plasmids

| Plasmid        | Description            | Reference or source |
|----------------|------------------------|---------------------|
| pZIS1          | Derivative of pZ152 (21a) carrying IS1 inserted between bla and tetA | 14                   |
| pMJ5           | Derivative of pZIS1 carrying insE-am4179 (C→T at IS1 bp 179) and A→G at IS1 bp 175 | 10                   |
| pMJ13          | Derivative of pZIS1 carrying insE-am328 (G→C at IS1 bp 328) | 10                   |
| pMJ73          | Derivative of pZIS1 carrying insE-am426 (G→A at IS1 bp 426) | M. Jakowec          |
| pMS1400        | IS1 insE-am328 BamHI-MscI fragment from pMJ13 and the BamHI-MscI backbone of pZIS1 | This work           |
| pMS1409        | IS1 insE-am328 MluI-ScaI fragment from pMS1400 and the MluI-ScaI backbone of pZIS1 | This work           |
| pOX38Km        | F-based conjugative plasmid carrying the kan gene from Tn9 | 3                    |
| pOX38Km finP   | finP derivative of pOX38Km | This work           |

TABLE 3. Bacterial strains

| Strain            | Genotype                          | Reference or source |
|-------------------|-----------------------------------|---------------------|
| Escherichia coli  |                                   |                     |
| GE999             | MC4100 ΔrecA1398 hocS20 (r− m−) recA13 ara14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44 | G. Weinstock        |
|                   |                                    | 15                   |
| HB101             |                                    | G. Weinstock        |
|                   |                                    | 2                    |
| MC4100            | F− araD139 Δ(argF-lac)J169 rpsL150 relA1 fbbB5301 deoC1 ptaF25 rbsR | G. Weinstock        |
|                   |                                    | 2                    |
| MS4086            | HB101 nal rif                      | 2                    |
| Salmonella typhi-|                                   |                     |
| murium LT2        |                                   |                     |
| MS1868            | leuA-am414 hocS20 (r− m−)          | 9                    |
| MS3371            | MS1868F′ lac finP                   | This work           |
| MS3371            | MS1868 str                         | 8                    |
| MS3371            | MS3371/pOX38Km finP                 | This work           |
| PY13761           | leuA-am414 hocS20 (r− m−)F′ ts114 lac zsf-20::Tn10 zsf-357::Mud-Q | 21                   |
| TP134             | leuA-am414 hocS20 recA             | 6                    |


**TABLE 4. Frequencies of cointegrates**

| Host and plasmid | IS1 | Frequency of cointegrates in**: recA** strains | recA strains |
|------------------|-----|---------------------------------------------|--------------|
| **E. coli**       |     |                                              |              |
| pBR322           | None | (<\(1.9 \pm 0.5\) \times 10^{-8} [4]         | (<\(4.1 \pm 0.6\) \times 10^{-8} [4]          |
| pZJ1S1           | Wild type | (3.2 \pm 1.2) \times 10^{-6} [4]          | (1.1 \pm 0.1) \times 10^{-6} [4]          |
| pMJ5             | insA-am179 | (6.8 \pm 2.3) \times 10^{-8} [4]          | (9.0 \pm 1.6) \times 10^{-8} [4]          |
| pMS1409          | insE-am328 | (1.0 \pm 0.3) \times 10^{-6} [7]          | (2.9 \pm 1.0) \times 10^{-7} [6]          |
| pMJ73            | insE-am426 | (7.8 \pm 1.8) \times 10^{-7} [4]          | (2.7 \pm 0.5) \times 10^{-6} [4]          |
| **S. typhimurium**|     |                                              |              |
| pBR322           | None | (5.6 \pm 2.0) \times 10^{-8} [5]          | (<\(3.2 \pm 1.4\) \times 10^{-8} [6]          |
| pZJ1S1           | Wild type | (5.7 \pm 0.7) \times 10^{-6} [5]          | (4.2 \pm 0.6) \times 10^{-6} [6]          |
| pMJ5             | insA-am179 | (6.2 \pm 1.8) \times 10^{-8} [5]          | (8.4 \pm 3.9) \times 10^{-8} [6]          |
| pMS1409          | insE-am328 | (3.3 \pm 0.4) \times 10^{-7} [5]          | (<\(1.2 \pm 0.3\) \times 10^{-6} [6]          |
| pMJ73            | insE-am426 | (4.9 \pm 0.7) \times 10^{-8} [5]          | (7.7 \pm 1.3) \times 10^{-6} [6]          |

* Donors were derivatives of MC4100, GE999, MS1868, and TP134 carrying pOX38Km \(fnp\) (Kan\(^{\text{r}}\)) and the indicated \(\text{Tet}^{\text{r}}\) plasmids. The recipient was \(E.\\ coli\) HB101 \(\text{nal n}^{\text{r}}\) (MS4086).

* The frequency of cointegrates is the number of \(\text{Tet}^{\text{r}}\) \(\text{Kan}^{\text{r}}\) \(\text{Na}^{\text{r}}\) \(\text{Rif}^{\text{r}}\) transconjugants (which occur by transfer of a cointegrate between pOX38Km \(fnp\) and the IS1 plasmid) divided by the number of \(\text{Kan}^{\text{r}}\) \(\text{Na}^{\text{r}}\) \(\text{Rif}^{\text{r}}\) transconjugants (which occur by transfer of pOX38Km \(fnp\)). The mean and standard error are given, and the number of measurements is in brackets.

* 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** 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 less than 10-fold in \(S.\\ typhimurium\) but had little effect in \(E.\\ coli\). In agreement with the results of Jakowee et al. (10), who reported that the activity of this mutant was normal in \(E.\\ coli\) recA** \(\text{thyA}^{\text{r}}\) but was reduced 70-fold in recA** \(\text{thyA}^{\text{r}}\). 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.

Jakowee 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 \(E.\\ coli\).

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