Role of the IS50R Proteins in the Promotion and Control of Tn5 Transposition

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IS50R, the inverted repeat sequence of Tn5 which is responsible for supplying functions that promote and control Tn5 transposition, encodes two polypeptides that differ at their N terminus. Frameshift, in-frame deletion, nonsense, and missense mutations within the N terminus of protein 1 (which is not present in protein 2) were isolated and characterized. The properties of these mutations demonstrate that protein 1 is absolutely required for Tn5 transposition. None of these mutations affected the inhibitory activity of IS50, confirming that protein 2 is sufficient to mediate inhibition of Tn5 transposition. The effects on transposition of increasing the amount of protein 2 (the inhibitor) relative to protein 1 (the transposase) were also analyzed. Relatively large amounts of protein 2 were required to see a significant decrease in the transposition frequency of an element. In addition, varying the co-ordinate synthesis of the IS50R proteins over a 30-fold range had little effect on the transposition frequency. These studies suggest that neither the wild-type synthesis rate of protein 2 relative to protein 1 nor the amount of synthesis of both IS50R proteins is the only factor responsible for controlling the transposition frequency of a wild-type Tn5 element in Escherichia coli.

1. Introduction

Tn5 is a composite transposon consisting of two inverted copies of IS50 flanking a 2.7 kb§ sequence of DNA which contains a gene conferring resistance to neomycin and kanamycin (see Fig. 1; Berg et al., 1975). Transposition of Tn5 involves the action of the Tn5-encoded transposase plus possible host factors at the termini of the element (Rothstein et al., 1980; Isberg & Syvanen, 1981; Johnson & Reznikoff, 1983). All of the Tn5-encoded functions that are required for transposition and its control are encoded on IS50R. Two polypeptides, protein 1 (P1) and protein 2 (P2), are synthesized from an open reading frame that extends most of the length of IS50R (Fig. 1). These two proteins share the same amino acid sequence, except that P1 has an additional 40 residues at its N terminus (Rothstein & Reznikoff.

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§ Abbreviations used: kb, 10^3 bases or base-pairs; bp, base-pairs; P1, protein 1; P2, protein 2; IPTG, isopropyl-β-D-thio-galactoside; SDS, sodium dodecyl sulfate.
FIG. 1. Genetic and physical map of Tn5. The nucleotides are numbered such that the terminal base-pair of the outer end of IS50 is +1. The wavy lines represent RNAs and the lower continuous lines designated proteins.

1981; Johnson & Reznikoff, 1981). P2 is synthesized about four times the level of P1, presumably due to differential translation initiation frequencies. The analogous proteins from IS50L are not functional in the promotion or control of transposition because of an ochre codon that terminates the IS50L proteins 26 amino acid residues prematurely (Auerswald et al., 1980; Rothstein & Reznikoff, 1981).

Insertion or deletion mutations within the coding sequence of IS50R eliminate the ability of the mutant element to transpose, and insertion mutations within the N terminus of P1 implicate this protein as being essential for transposase activity (Rothstein et al., 1980; Isberg et al., 1982). The transposase activity is also distinguished by its inability to act efficiently in trans (Isberg & Syvanen, 1981; Isberg et al., 1982; Johnson et al., 1982). Transposition defective mutants can only be efficiently complemented to transpose when IS50R functions are supplied in the immediate vicinity, either by suppression of the ochre allele of IS50L or by placing a functional IS50R donor close by on the same replicon (Rothstein & Reznikoff, 1981; Johnson & Reznikoff, 1983).

IS50R also encodes a trans-acting inhibitor of Tn5 transposition (Biek & Roth, 1980). This inhibitory activity has been studied in *Escherichia coli* by two different assays. In the "infection" assay, multiple copies of IS50R were shown to inhibit the subsequent transposition of Tn5 when introduced into the cell on an infecting phage DNA molecule (Isberg et al., 1982; Johnson et al., 1982). In the "copy number control" assay, the transposition of mono-copy Tn5 was inhibited in the presence of multiple copies of Tn5 (IS50R). This control over transposition in the steady-state results in a constant overall transposition frequency in a cell regardless of the number of Tn5 elements present (Johnson & Reznikoff, 1984). P2 was found to be sufficient to mediate the inhibition in both of these assays, and the amount of inhibition was shown to be proportional to the amount of P2 synthesized. The inhibition was not a result of P2 regulating the synthesis of the IS50R proteins, and so it has been proposed that P2 is directly inhibiting the transposition reaction (Isberg et al., 1982; Johnson et al., 1982).

In this paper, we have analyzed in detail the role of the unique N terminus of
IS50R PROTEINS AND Tn5 TRANSPOSITION

P1 in transposase activity by characterizing deletion, frameshift, nonsense, and missense mutations within this region. We have also investigated the effect on the transposition frequency of an element when the co-ordinate or differential synthesis of the IS50R proteins is varied in order to gain some insight as to the role of P1 and P2 in modulating the Tn5 transposition frequency. To our surprise, the normal synthesis rate of the IS50R proteins in E. coli does not seem to be directly responsible for controlling the rate of transposition of a Tn5 element, leading us to propose that cellular functions may be limiting the maximum rate of transposition.

2. Materials and Methods

(a) Growth media, supplies, and general techniques

Bacteria were grown in LB (rich), M9 plus 0.4% (w/v) glucose, or superbroth (Davis et al., 1980). Antibiotics (Sigma) were used at the following concentrations: 25 or 40 μg kanamycin/ml (corrected for potency), 5 or 15 μg tetracycline/ml, and 100 μg ampicillin/ml. Restriction enzymes were purchased from Bethesda Research Laboratories or New England Biolabs. Bacteriophage T4 DNA polymerase, S1 nuclease, and phage T4 polynucleotide kinase were from PL Biochemicals; the large fragment of DNA polymerase I was from Promega Biotec Inc. Phage T4 DNA ligase and Micrococcus luteus DNA polymerase were gifts of R. Simoni and R. D. Wells, respectively. Bachem Chemicals supplied IPTG (isopropyl-β-d-thio-galactoside). The basic recombinant DNA techniques and transformation procedures are as described (Davis et al., 1980).

(b) Bacterial strains and plasmids

Table 1 summarizes the genotypes or structures of the E. coli strains and plasmids used in this study. The ochre suppressing strains are all derived from the isogenic set of nonsense suppressors described by Miller et al. (1977). RZ427, RZ432, RZ437 and RZ448 were obtained by co-transducing srl : : Tnl0 and recA56 using Plcm into the appropriate suppressor strain followed by selecting fusaric acid-resistant, tetracycline-sensitive isolates (Bochner et al., 1980). All of the plasmids are derived from ColEl or pBR322. The number of the Tn5 mutant is derived from the plasmid number. For instance, Tn5-102, which is wild-type Tn5, is contained on pRZ102. Further details of plasmid constructions and structures are given below and in Results.

(c) Construction of pRZ423 and pRZ424

pRZ236 is a pBR322-derived plasmid that contains a functional IS50R adjacent to the neo gene of Tn5 (Rothstein & Reznikoff, 1981). An EcoRI + SalI digest of pRZ236 and pRZ149 was ligated together and a col' Kan' (5 μg/ml) transformant was selected to give pRZ421. The 2.8 kb BglII restriction fragment containing the tetracycline resistance determinant from Tf10 was ligated into the BamHI site of pRZ421 to give pRZ423. The construction of pRZ423 was identical to pRZ423, except that pRZ401 (see below) was substituted for pRZ149. pRZ190 is wild-type Tn5 containing the tetracycline resistance determinant. The orientation of the tetracycline resistance determinant relative to the neo gene in pRZ190, pRZ423 and pRZ424 is the same.

(d) Construction of pRZ382

The lacUV5 promoter and operator was substituted for the IS50R inverted repeat promoter in the following manner. The lacPU5 95 bp fragment containing BamHI and
### Table 1

**Bacterial strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Relevant markers/structure</th>
<th>Source/derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hfl-1</td>
<td>hfl-1 ser trp leu ile rpsL</td>
<td>Belfort &amp; Wulff (1971)</td>
</tr>
<tr>
<td>LE392</td>
<td>supE supF</td>
<td>L. Enquist</td>
</tr>
<tr>
<td>BD1528</td>
<td>ung-1 nadB7 met supE supF</td>
<td>B. Duncan <em>via</em> D. Shortle</td>
</tr>
<tr>
<td>XA10B</td>
<td>SupB Δ(pro-lac) argEam metB ara</td>
<td>Miller et al. (1977)</td>
</tr>
<tr>
<td>RZ102</td>
<td>F' recA56 rpsL</td>
<td>Johnson et al. (1982)</td>
</tr>
<tr>
<td>RZ104</td>
<td>pOX38 : : Tn5-191/RZ102</td>
<td>Johnson &amp; Reznikoff (1983)</td>
</tr>
<tr>
<td>RZ201</td>
<td>Δ(pro-lac) ara rpsL</td>
<td>Johnson et al. (1982)</td>
</tr>
<tr>
<td>RZ221</td>
<td>polA Δ(pro-lac) ara rpsL</td>
<td>T. McNeil &amp; W. Reznikoff</td>
</tr>
<tr>
<td>RZ427</td>
<td>supC recA56 srl Δ(pro-lac) argEam ara nalA rif</td>
<td>This work</td>
</tr>
<tr>
<td>RZ432</td>
<td>supB recA56 srl Δ(pro-lac) argEam ara nalA rif</td>
<td>This work</td>
</tr>
<tr>
<td>RZ434</td>
<td>F' prolac1*Zau18/+supB recA56 srl Δ(pro-lac) argEam ara nalA rif</td>
<td>This work</td>
</tr>
<tr>
<td>RZ437</td>
<td>supC' recA56 srl Δ(pro-lac) argEam ara nalA rif</td>
<td>This work</td>
</tr>
<tr>
<td>RZ448</td>
<td>sup'' recA56 srl Δ(pro-lac) argEam ara nalA rif</td>
<td>This work</td>
</tr>
<tr>
<td>RZ508</td>
<td>pOX38-Gen/BD1528 recA56 srl : : Tn10</td>
<td>transfer pOX38-Gen (Johnson &amp; Reznikoff, 1983a,b) by conjugation</td>
</tr>
</tbody>
</table>

- **pRZ102**
  - Jorgensen et al. (1979)
- **pRZ112**
  - Jorgensen et al. (1979)
- **pRZ149**
  - Rothstein et al. (1980)
- **pRZ174**
  - Rothstein et al. (1980)
- **pRZ305**
  - Johnson et al. (1982)
- **pRZ312**
  - This work
- **pRZ382**
  - This work
- **pRZ384**
  - This work
- **pRZ405-pRZ407**
  - This work
- **pRZ413**
  - This work
- **pRZ414**
  - This work
- **pRZ190**
  - S. Rothstein & W. Reznikoff
- **pRZ423**
  - This work
- **pRZ424**
  - This work
- **pRZ436-pRZ442, pRZ445**
  - Sodium bisulfite mutagenesis of pRZ414, regenerate functional IS50L
- **pRZ368**
  - Hydroxylamine mutagenesis of pRZ102
  - Y. D. Zhang & W. Reznikoff

The structure of the Tn5 DNA is diagrammed where appropriate. Filled bars: DNA from IS50R. Open bars: DNA from IS50L. Hatched bars: deleted DNA. Δ indicates a small deletion.
SalI restriction sites was cloned from pRW28 (Klein & Wells, 1982) into the BamHI and SalI sites of pRZ344 (R. Johnson & W. Reznikoff, unpublished results). This placed the lacPO adjacent to the other 56 bp of IS50L. An EcoRI-HindIII (SalI) fragment that contained the outer 56 bp of IS50L and the lacPO was ligated into the EcoRI and HpaI sites of pRZ204 (Rothstein & Reznikoff, 1981) to give pRZ377. The modified IS50R from pRZ377 was then substituted for the IS50R in pRZ143 (Rothstein et al., 1980) using the EcoRI and SalI sites to give an intact Tn5 structure (pRZ382) with IS50R containing the lacPO controlling the expression of protein 2.

(e) β-Galactosidase fusions

The construction of pRZ305, which has the last amino acid of IS50R fused to the 8th amino acid of β-galactosidase, has been described. This fusion was transferred into pRZ405-pRZ407 using the HindIII and SalI restriction sites and into pRZ382 by replacing its internal HindIII fragment with that of pRZ305 to give pRZ384. pRZ312 is the analogous C-terminal lacZ fusion to IS50L and was constructed in a similar manner as pRZ305, except that the fusion was initially made to the filled-in BglII site of pRZ201 (a pBR322-derived plasmid containing IS50L; Rothstein & Reznikoff, 1981), and the Lac' transformants were selected in XA10B (supB) to allow for suppression of the IS50L ochre allele.

(f) S1-generated deletions

pRZ149, which contains the right half of Tn5 (IS50R) in ColEI, was linearized at its unique HpaI restriction site and subjected to S1 nuclease at 1 unit of DNA/µg in 25 mM-sodium acetate (pH 4-6), 25 mM-NaCl, and 3 mM-ZnSO4. After 15 min at 30°C, the reaction was terminated by the addition of EDTA and diethyl pyrocarbonate to a final concentration of 10 mM and 0.05% (w/v), respectively. After dialysis and precipitation with ethanol, the DNA was re-circularized in the presence of T4 DNA ligase and transformed into RZ201. The exact endpoints of the deletions were determined by the DNA sequencing methods of Maxam & Gilbert (1980) using DNA 32P-labeled at the 5' end of the RsaI site (nucleotide 173) by polynucleotide kinase and [γ-32P]ATP. Deletions removing 1, 17 and 18 bp (pRZ401-pRZ403) were saved for further analysis. IS50L was added to the deletion mutants regenerating an intact Tn5 structure by ligating an EcoRI + SalI digest of pRZ201 and pRZ401-pRZ403 and selecting Col' Kan' transformants giving pRZ405-pRZ407, respectively. pRZ413 is pRZ407 containing the Tn5-174 insertion mutation at the HindIII site. It was constructed by ligating an XhoI digest of pRZ403 with a XhoI + PvuI (which cuts ColEI at 2 sites) of Tn5-175 (Rothstein et al., 1980) and selecting a transformant that contained the HindIII insertion but was missing the HpaI site (pRZ411). IS50L was recloned into pRZ411 as described above to give pRZ413.

(g) Localized mutagenesis with sodium bisulfite

The 3' exonuclease activity of T4 DNA polymerase was used to generate single-stranded regions extending on either side of the HpaI site of IS50R. For these 3' exonuclease digestions, 2.5 units of T4 DNA polymerase were added per µg of HpaI-digested pRZ414 in 33 mM-Tris-acetate (pH 7.9), 66 mM-potassium acetate, 10 mM-magnesium acetate, 500 µM-dithiothreitol, 500 µM-dATP. After 2 to 5 min at 37°C, the reaction was terminated by the addition of diethyl pyrocarbonate to a final concentration of 0.05%. Apparently, the T4 DNA polymerase did not efficiently terminate its 3'→5' digestion at single adenosine residues as might be expected. However, it did not appear to proceed through the tandem adenosine residues at nucleotides 132 to 134 and 204-205 as judged from the resulting mutations (see below). The conditions for the sodium bisulfite reactions and the following dialysis were as described by Shortle & Nathans (1978), except that the bisulfite reactions were incubated at 37°C for only 15 min. The single-stranded
region was filled in using *M. luteus* DNA polymerase in the presence of 25 mM-dNTPs for 2 h at 22°C in 20 mM-Tris - HCl (pH 7.6), 10 mM-MgCl \(_2\), 1 mM-dithiothreitol followed by the addition of T4 DNA ligase and ATP to 100 μM and incubation overnight at 15°C. The DNA was then transformed into a strain deficient for uracil glycosylase to prevent repair of the deoxyuracil residues *in vivo* and the transformants were screened for a reduction in the transposition proficiency of the Tn5 by a mating out assay or for loss of the *HpaI* site.

Approximately 30% of the transformants were defective for transposition and 8 mutants were characterized that contained different point mutations within the unique 40 amino acid residues of P1. The DNA sequence and resulting amino acid changes are shown in Fig. 3. All of the nucleotide changes are consistent with a sodium bisulfite-induced cytosine to uracil transition in the exposed single-stranded regions, and all were contained within the stretch of DNA between the tandem adenine residues at nucleotides 132 to 134 to 204-205. While a number of the mutants contained double mutations, only 2 resulted in a change of more than 1 amino acid.

(h) DNA sequence analysis of point mutations

For DNA sequencing, an EcoRI-XhoI restriction fragment from the mutant plasmids was ligated into the *EcoRI* and *SalI* sites of M13 mp8. An *HaeIII*-NarI restriction fragment (nucleotides 373 to 327) was used as a primer for the dideoxy-sequencing reactions of Sanger et al. (1977), and the DNA from less than nucleotide 100 to greater than nucleotide 300 on IS50R was read for each mutant. The mutant IS50Rs were re-cloned into an intact Tn5 (IS50R') for all the assays shown in this paper.

(i) Transposition assays

Transposition proficiency was quantitated by the λ induction assay (Rothstein et al., 1980; Johnson et al., 1982). In brief, λc1857 b315 b319 nin5 Sam7 (λbn) lysogens containing Tn5 were grown in superbroth or M9-glucose +0.2% Casamino acids (+1 mM-IPTG, where appropriate, and the λ prophage was thermostated at mid-exponential growth phase. The resulting lysate was used to transduce Hfl1 to kanamycin or tetracycline resistance and the number of antibiotic-resistant colonies was compared to the number of plaque forming units of the phage (on LE392) to give the transposition frequency.

The mating-out assay used to screen for transposition defective mutants after sodium bisulfite mutagenesis was similar to that described by Johnson & Reznikoff (1984). Kanamycin-resistant transformants of RZ508 were mated with RZ221 and isolates that gave a reduced frequency of Kan’, Gen’ and Nal‘ exconjugants were analyzed further.

(j) Tn5 regulation assays

The λ infection assays were performed in RZ102 using λc1857 b221 Oam29 Pam80 rec::Tn5 as described by Johnson et al. (1982). The copy number control assay measures the inhibition of transposition into λbn of the single copy Tn5-191 element in RZ104λbn by multiple copies of the test transposon (Johnson & Reznikoff, 1984).

(k) β-Galactosidase assays

 Cultures were grown in M9-glucose +0.2% Casamino acids, with 1 mM-IPTG where appropriate, and assayed as described by Miller (1972).

(l) Immunoprecipitation of Tn5-lacZ fusion proteins

The conditions for labeling cells with [35S]methionine, preparation of extracts, and immunoprecipitations with anti-β-galactosidase antibody were as described (Johnson et al., 1982). Sodium dodecyl sulfate/polyacrylamide gel electrophoresis was done according to Laemmli (1970).
Fig. 2. Immunoprecipitation of Tn5-lac fusions containing mutations of protein 1. Shown is the autoradiograph of a 5% polyacrylamide gel of the immunoprecipitates obtained from RZ201 containing pRZ305 (IS50R-lacZ), pRZ405-lacZ (an in-frame deletion of protein 1), pRZ406-lacZ, and pRZ407-lacZ (out-of-frame deletions of protein 1), and pRZ445-lacZ (an ochre mutation of protein 1). The IS50R fusion protein bands are labeled P1-β-gal and P2-β-gal.

3. Results

(a) Mutations in the N terminus of protein 1

Previous studies have suggested that the N-terminal portion of P1 is essential for the transposase activity of this protein, while P2, which lacks this sequence, acts as an inhibitor of Tn5 transposition. Mutations for the study of this critical portion of P1 were isolated by three different procedures. (1) Short deletions were constructed at the HpaI restriction site of IS50R using the double-stranded nibbling activity of S1 nuclease (see Materials and Methods). DNA sequence
analysis revealed that Tn5-406 and Tn5-407 contained out-of-frame deletions of 1 and 17 bp, while Tn5-405 contained an in-frame deletion of 18 bp, removing six amino acid residues from P1. The presence of the frameshift and internal deletions was confirmed by fusing lacZ to the C terminus of the mutant IS50R proteins and immunoprecipitating the fusion proteins with anti-β-galactosidase antibody. SDS/polyacrylamide gel electrophoresis of the immunoprecipitates demonstrated that P1 was not synthesized in Tn5-406 or Tn5-407 but a polypeptide corresponding to the mutant P1 was present in Tn5-405 (Fig. 2). Each of the mutants synthesized normal amounts of P2.

(2) Point mutations were generated by a scheme described in Materials and Methods. The single-strand-specific mutagen sodium bisulfite was used to generate C → U(T) transitions in a single-stranded region formed by the 3' → 5' exonuclease activity of T4 DNA polymerase acting at the HpaI site of IS50R. The resulting mutations (Tn5-436 to Tn5-445) are described in Figure 3. The presence of the ochre mutation in Tn5-445 was demonstrated by immunoprecipitating the IS50R-encoded proteins when they were fused to lacZ (Fig. 2). P1 is not made by Tn5-445, as predicted, and P2 synthesis appears normal.

(3) The five hydroxylamine-induced transposition-defective mutants described
previously (Y.-D. Zhang & W. Reznikoff, unpublished results; Johnson et al., 1982) were screened by subcloning experiments for their possible location in the region. Tn5-368 was found to contain a mutation between the HpaI and the XhoI restriction sites of IS50R. When the DNA within this region was sequenced, a C/G to T/A transition was found at nucleotide 226 (Fig. 3), which changes a serine to leucine at the 30th amino acid of P1. The mutations responsible for the transposition defect in the remaining four mutants are located at different sites within the common coding sequence of P1 and P2.

Transposition assays revealed that none of the deletion mutations (including the in-frame 6 amino acid deletion Tn5-405) is able to transpose (Table 2). They behaved the same as the previously characterized transposition-defective mutant, Tn5-174, which contains an insertion mutation at the HindIII site of IS50R inactivating both IS50R proteins (Rothstein et al., 1980). Therefore, P1 must be absolutely required for transposition; P2 is not sufficient to mediate the reaction. With the exception of Tn5-438, each of the point mutations in the N terminus of P1 has a severe effect on transposition (Fig. 3). The Tn5 mutants containing amino acid changes as residues 5, 9, 15, 20 and 30, and an ochre mutation at residue 22 of P1 are completely defective for transposition. These mutations demonstrate clearly the absolute importance of the first 30 amino acids of P1 for transposase activity. The valine to isoleucine change at amino acid 17 of P1 in Tn5-438 does not appear to significantly affect the transposase activity (Tn5-438 was selected for analysis because it had lost the HpaI site).

Mutants containing deletion, frameshift and missense mutations of P1 were assayed for their ability to inhibit transposition of Tn5 by two assays. The data in Table 3 indicate that all of the mutants are able to effectively inhibit the transposition of an infecting Tn5. In addition, multiple copies of P1-defective Tn5s are able to inhibit the transposition of a mono-copy Tn5 as efficiently as the wild-type element (Table 3). These results demonstrate that P2 is sufficient to provide the inhibitory activity and further demonstrates that the element does not have to be transposition proficient to mediate copy number control or to inhibit the transposition of an infecting Tn5.

### Table 2

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sequences deleted</th>
<th>Number of nucleotides deleted</th>
<th>Resulting mutation*</th>
<th>Transposition frequencies</th>
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</thead>
<tbody>
<tr>
<td>Tn5-102</td>
<td>None</td>
<td></td>
<td>Wild-type</td>
<td>4.5 x 10^-6</td>
</tr>
<tr>
<td>Tn5-114</td>
<td>Insertion at 1199</td>
<td></td>
<td>Protein 1 &amp; 2 inactive</td>
<td>&lt;2.2 x 10^-8</td>
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<tr>
<td>Tn5-405</td>
<td>(177-179)-(195-197)</td>
<td>18</td>
<td>6 amino acid deletion</td>
<td>&lt;1.7 x 10^-8</td>
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<tr>
<td>Tn5-406</td>
<td>180</td>
<td></td>
<td>Frameshift</td>
<td>&lt;1.2 x 10^-8</td>
</tr>
<tr>
<td>Tn5-407</td>
<td>(179-181)-(196-198)</td>
<td>17</td>
<td>Frameshift</td>
<td>&lt;1.3 x 10^-8</td>
</tr>
</tbody>
</table>

* With respect to protein 1; protein 2 is unaltered except in Tn5-174.

b Values represent transpositions per plaque-forming unit in λ induction assays using RZ102bha.

c Compares the transposition frequency relative to Tn5-102.
### Table 3

**Regulation assays on protein 1 defective mutants**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>IS50R mutation</th>
<th>Transposition (p.f.u.)</th>
<th>Copy number control assay (p.f.u.)</th>
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<tbody>
<tr>
<td>ColEl</td>
<td>Control</td>
<td>3.3 × 10^{-4}</td>
<td>4.8 × 10^{-6}</td>
</tr>
<tr>
<td>Tn5-102</td>
<td>Wild-type</td>
<td>9.0 × 10^{-6}</td>
<td>3.4 × 10^{-7}</td>
</tr>
<tr>
<td>Tn5-406</td>
<td>Protein 1 frameshift</td>
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<td>1.2 × 10^{-7}</td>
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<tr>
<td>Tn5-407</td>
<td>Protein 1 frameshift</td>
<td>1.2 × 10^{-5}</td>
<td>1.5 × 10^{-7}</td>
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<td>7.5 × 10^{-6}</td>
<td>2.6 × 10^{-7}</td>
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<td>2.7 × 10^{-7}</td>
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<tr>
<td>Tn5-440</td>
<td>Protein 1 missense</td>
<td>1.3 × 10^{-6}</td>
<td>2.2 × 10^{-7}</td>
</tr>
<tr>
<td>Tn5-441</td>
<td>Protein 1 missense</td>
<td>1.5 × 10^{-6}</td>
<td>1.9 × 10^{-7}</td>
</tr>
<tr>
<td>Tn5-442</td>
<td>Protein 1 missense</td>
<td>9.0 × 10^{-6}</td>
<td>3.4 × 10^{-7}</td>
</tr>
</tbody>
</table>

*a* Values represent the number of kanamycin-resistant colonies per plaque-forming unit of λ::Tn5. For the infection assays, the cells contained multiple copies of the test IS50R cloned into pBR322. % compares the transposition frequency relative to cells containing ColEl.

*b* Values represent the number of Tn5-191 transpositions per plaque-forming unit of the phage. % compares the frequency of Tn5-191 in the presence of multiple copies of the mutant Tn5 with the uninhibited transposition frequency of Tn5-191 (ColEl).

Complementation studies were performed on the Tn5 element containing the presumed P1 mutations to confirm that the defect in these mutants was due to the altered protein and not the result of a structural or some other cis-dominant mutation. Efficient complementation of insertion or deletion mutations in the common coding sequence for P1 and P2 can be achieved by suppressing the IS50L ochre allele by supB (Rothstein & Reznikoff, 1981; Johnson et al., 1982). Table 4 shows that the deletion, frameshift and missense mutations are all capable of

### Table 4

**cis-complementation of protein 1 defective mutants**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>IS50R mutation</th>
<th>Transposition frequency</th>
<th>Transposition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn5-102</td>
<td>Wild-type</td>
<td>1.5 × 10^{-5}</td>
<td>100</td>
</tr>
<tr>
<td>Tn5-174</td>
<td>Protein 1 &amp; 2 insertion</td>
<td>6.6 × 10^{-6}</td>
<td>44</td>
</tr>
<tr>
<td>Tn5-406</td>
<td>Protein 1 frameshift</td>
<td>3.5 × 10^{-6}</td>
<td>23</td>
</tr>
<tr>
<td>Tn5-407</td>
<td>Protein 1 frameshift</td>
<td>2.7 × 10^{-6}</td>
<td>18</td>
</tr>
<tr>
<td>Tn5-413</td>
<td>Protein 1 frameshift + protein 2 insertion</td>
<td>6.1 × 10^{-6}</td>
<td>41</td>
</tr>
<tr>
<td>Tn5-405</td>
<td>Protein 1 deletion</td>
<td>3.2 × 10^{-6}</td>
<td>21</td>
</tr>
<tr>
<td>Tn5-436</td>
<td>Protein 1 missense</td>
<td>3.7 × 10^{-6}</td>
<td>25</td>
</tr>
<tr>
<td>Tn5-437</td>
<td>Protein 1 missense</td>
<td>2.3 × 10^{-6}</td>
<td>15</td>
</tr>
<tr>
<td>Tn5-439</td>
<td>Protein 1 missense</td>
<td>4.2 × 10^{-6}</td>
<td>28</td>
</tr>
<tr>
<td>Tn5-440</td>
<td>Protein 1 missense</td>
<td>3.3 × 10^{-6}</td>
<td>22</td>
</tr>
<tr>
<td>Tn5-441</td>
<td>Protein 1 missense</td>
<td>2.7 × 10^{-6}</td>
<td>18</td>
</tr>
<tr>
<td>Tn5-442</td>
<td>Protein 1 missense</td>
<td>3.8 × 10^{-6}</td>
<td>25</td>
</tr>
</tbody>
</table>

*a* Transpositions per plaque-forming unit of the phage assayed in RZ432bbn.

*b* Compares the transposition frequency relative to Tn5-102.
IS50R PROTEINS AND Tn5 TRANSPOSITION

being complemented to transpose, indicating that the defect in all of these mutants is the aberrant P1.

It should be noted that the complementation efficiency of all of these mutants is significantly lower than Tn5-174, which contains an insertion in the IS50R HindIII site inactivating both P1 and P2. This reduced level of complementation is probably due to the greater amount of functional inhibitor (P2) that is synthesized by the P1-defective mutants under these conditions (see below). This was demonstrated specifically for Tn5 407. Introduction of an insertion into the HindIII site of IS50R in Tn5-407 (thereby generating Tn5-413) results in a construct that would not overproduce P2 relative to P1. Its transposition frequency is equivalent to Tn5-174 (Table 4).

(b) Effect of the ratio of protein 1 to protein 2 on transposition

The ability of the P1-deficient mutants to be complemented to transpose at a reasonably high frequency by suppression of IS50L is somewhat surprising, since these mutants should be synthesizing very large amounts of the inhibitor protein (P2) relative to the transposase protein, P1. The approximate ratio of the functional IS50R proteins synthesized by these mutants in the presence of supB can be estimated to be about 24 monomers of P2 to one monomer of P1. This estimate is obtained by assuming that the suppression of the IS50L ochre allele is 20% of the IS50R synthetic rate (see below) and that the normal ratio of the IS50R proteins is four monomers of P2 to one monomer of P1 (Johnson et al.,

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Structure</th>
<th>Protein 1</th>
<th>Protein 2</th>
<th>Transposition frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn5-190</td>
<td>tet1</td>
<td>1</td>
<td>4</td>
<td>4.1 x 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>tet1</td>
<td>2</td>
<td>8</td>
<td>3.8 x 10^{-6}</td>
</tr>
<tr>
<td>Tn5-424</td>
<td>tet1</td>
<td>1</td>
<td>8</td>
<td>4.0 x 10^{-6}</td>
</tr>
</tbody>
</table>

* The schematic structure of the Tn5 mutant is shown along with the functional IS50R proteins that are synthesized.

* Assumes one copy of IS50R synthesizes 1 monomer of protein 1 or 4 monomers of protein 2 (Johnson et al., 1982).

* Transpositions per plaque-forming unit by the λ induction assay in RZ102λbba containing the Tn5 mutants located on ColE1 plasmids.

* Compares the transposition frequency to that of Tn5-190 (wild-type Tn5 conferring tetracycline resistance).

TABLE 5

Effect of changing IS50R protein expression on transposition

---

<table>
<thead>
<tr>
<th>Protein 1</th>
<th>Protein 2</th>
<th>Transposition frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4.1 x 10^{-6}</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>3.8 x 10^{-6}</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>4.0 x 10^{-6}</td>
</tr>
</tbody>
</table>

* The schematic structure of the Tn5 mutant is shown along with the functional IS50R proteins that are synthesized.

* Assumes one copy of IS50R synthesizes 1 monomer of protein 1 or 4 monomers of protein 2 (Johnson et al., 1982).

* Transpositions per plaque-forming unit by the λ induction assay in RZ102λbba containing the Tn5 mutants located on ColE1 plasmids.

* Compares the transposition frequency to that of Tn5-190 (wild-type Tn5 conferring tetracycline resistance).
This implies that there is about a six times higher ratio of P2 synthesized than functional P1 by the P1-defective mutants as compared to the normal ratio. However, the P1-defective mutants are able to transpose at about 50% of the rate of Tn5-174, which synthesizes functional IS50R proteins only via suppression of IS50L.

The low sensitivity of the transposition frequency to the changed ratio of inhibitor protein (P2) to transposase (P1) seen in the above experiment has been confirmed by two other types of experiments.

In one of the experiments described in Table 5, the gene dosage of the sequences encoding P2 was doubled relative to those encoding P1 (this is presumably reflected in a doubling of the P2 to P1 synthetic ratio) with no effect on the transposition frequency. This was accomplished by substituting a mutant IS50R that synthesizes only P2 for IS50L (see Tn5-424 versus Tn5-190 in Table 5).

The other experiment is described in Table 6. The Tn5-382 derivative allowed us to systematically vary the synthesis of P2 over a wide range relative to a constant amount of P1. Tn5-382 contains the normal IS50L for one inverted repeat, which will give a low level of functional IS50R proteins in a supB background, while the other repeat contains the lac promoter-operator controlling the expression of P2. The amount of expression of the proteins from each repeat was approximated by fusing the C terminus of these proteins to lacZ and assaying the amount of β-galactosidase expression from the fusion proteins (see footnote d to Table 6).

The results in Table 6 demonstrate that only under very high cellular synthetic ratios of P2 to P1 does the transposition rate decrease. At a ratio of approximately 30 monomers of P2 to one monomer of P1, only a 50% decrease in transposition occurs over the wild-type frequency. This ratio is similar to that calculated for the P1-defective mutants under suppressing conditions, where the transposition frequency was about 50% of Tn5-174 (Table 4). When over 60 times more monomers of P2 are synthesized than of P1, transposition is inhibited to about 7-5% of the frequency measured in the absence of any overproduction of P2. These results indicate that the synthetic ratio of the IS50R proteins can be important in modulating the transposition frequency of an element but relatively large amounts of P2 to P1 are required to see a significant inhibitory effect.

(c) Effect of varying the co-ordinate expression of IS50R proteins

The data in Tables 5 and 6 suggest that differences in the co-ordinate expression of P1 and P2 have little effect on the transposition rate of an element. The relevant experiment in Table 5 compares the transposition frequency of Tn5-423 in which both arms of Tn5 are IS50R (presumably doubling the synthesis of P1 and P2) to Tn5-190 (one copy of IS50R). The transposition frequency of Tn5-423 is the same as Tn5-190. In Table 6, the important experiment examines the effect of a sixfold change in co-ordinate synthesis of P1 and P2 (Tn5-102 versus Tn5-382, fully repressed). Again, no significant effect was observed.

The consequences of altering the co-ordinate expression of P1 and P2 was investigated in greater detail by analyzing the effects on the transposition
### Table 6

**Effect of changing IS50R protein expression on transposition**

<table>
<thead>
<tr>
<th>Mutant*</th>
<th>Host</th>
<th>Structure</th>
<th>Monomers of IS50R proteins</th>
<th>Ratio of IS50R proteins</th>
<th>Transposition frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protein 1</td>
<td>Protein 2</td>
<td>Protein 1</td>
</tr>
<tr>
<td>Tn5-102</td>
<td>I*</td>
<td></td>
<td>114</td>
<td>456</td>
<td>1</td>
</tr>
<tr>
<td>Tn5-382</td>
<td>Iq</td>
<td></td>
<td>18</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>Tn5-382</td>
<td>Iq + IPTG</td>
<td></td>
<td>18</td>
<td>531</td>
<td>1</td>
</tr>
<tr>
<td>Tn5-382</td>
<td>I^-</td>
<td></td>
<td>18</td>
<td>1182</td>
<td>1</td>
</tr>
</tbody>
</table>

* The Tn5 mutants were located on ColEl. Tn5-102 is wild-type Tn5.

* The assays were performed in RZ434 (F*pro-lacIqZaeIII[Δ(lac-pro) supE]abbn (I*) ± 1 mM-IPTG or RZ432abbn (I^-), which is isogenic with RZ434 except for the absence of the episome.

* The schematic structure of the Tn5 mutant is shown along with the functional IS50R proteins that are synthesized.

* The calculated number of monomers of the IS50R proteins per cell determined by β-galactosidase assays of the appropriate host containing pRZ308, pRZ312 or pRZ384 is given. The β-galactosidase units were multiplied by 3 to estimate the number of monomers of fusion proteins (Kelley & Yanofsky, 1982). These estimates assume that the β-galactosidase activity accurately reflects the amount of functional P1 and P2, which may not be entirely correct if the 2 fusion proteins have different specific activities or stabilities. Nonetheless, a comparison of the relative levels of β-galactosidase activity should reflect the relative levels of P1 and P2 synthesis.

* The calculated ratio of the IS50R proteins per cell for each assay culture. The values do not necessarily reflect the ratios per Tn5 element, since protein 1 acts preferentially on the element from which it was synthesized.

* Transpositions per plaque-forming unit (p.f.u.) of the phage by the λ induction assay.

* Relates the transposition frequency to that of Tn5-102.
### Table 7

<table>
<thead>
<tr>
<th>Host</th>
<th>Tn5 element</th>
<th>Transposition frequency (p.f.u.)</th>
<th>Transposition frequency (%)</th>
<th>β-Galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>sup0</td>
<td>Tn5-102</td>
<td>9.4 x 10^-6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Tn5-112</td>
<td>2.5 x 10^-8</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>supB</td>
<td>Tn5-102</td>
<td>6.0 x 10^-6</td>
<td>118.5</td>
<td>118.5</td>
</tr>
<tr>
<td></td>
<td>Tn5-112</td>
<td>4.2 x 10^-6</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>supC</td>
<td>Tn5-102</td>
<td>6.3 x 10^-6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Tn5-112</td>
<td>4.2 x 10^-6</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>supG</td>
<td>Tn5-102</td>
<td>4.5 x 10^-6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Tn5-112</td>
<td>3.0 x 10^-6</td>
<td>67</td>
<td>67</td>
</tr>
</tbody>
</table>

*a Tn5-102 is wild-type Tn5 containing IS50R and IS50L. Tn5-112 is a deletion mutant of Tn5 that contains only IS50L (see Table 1). They are located on ColEl.

*b Transpositions per plaque-forming unit (p.f.u.) of the phage assayed in RZ448 (sup0), RZ432 (supB), RZ437 (supC), and RZ427 (supG).

*c Compares the transposition frequency of Tn5-112 to Tn5-102 in the same host.

*d β-Galactosidase values are expressed relative to Tn5-102 in the same host. The value for Tn5-112 (IS50L) was measured using pRZ312 (IS50L-lacZ fusion). The value for Tn5-102 is the sum of the activities of pRZ305 (IS50R-lacZ fusion) and pRZ312. pRZ312 gave 2% of the activity of pRZ305 is RZ448, which was subtracted from each of the pRZ312 values.

As shown in Table 7, the suppression efficiency of the IS50L ochre codon varies with the particular tRNA suppressor from about 6% of the wild-type level for supC to about 20% of the wild-type level for supB. The calculated number of IS50R proteins per cell ranges from approximately 30 monomers (Tn5-112, supG) to 570 monomers (Tn5-102, supB). Nevertheless, little change is seen in the transposition frequencies of Tn5-112 versus Tn5-102 (wild-type) with these different suppressors. These results are explained most easily by a model in which variations in the co-ordinate synthesis of functional IS50R proteins does not affect the transposition frequency. Alternatively, the various amino acid substitutions may have differing effects on the activity or stability of the transposase and inhibitory functions, although such compensatory effects must yield virtually identical transposition frequencies for the three different amino acid substitutions.

### 4. Discussion

Previous studies have implicated the IS50R-encoded protein l as being absolutely required for transposition. Mutants of Tn5 that contained either the lacZ α-peptide fused to the N terminus of P1 or an insertion of DNA containing nonsense codons into the N-terminal coding sequence of P1 were unable to...
transpose (Rothstein et al., 1980; Isberg et al., 1982). In this study, frameshift, in-frame deletion, nonsense and missense mutations within the unique coding sequence of P1 that abolished the ability of the elements to transpose were isolated. The P1-defective mutants can be complemented in cis by suppression of IS50L, confirming that the defects are due to the mutant proteins. These results demonstrate clearly that P1 is absolutely required for transpositions and that its N-terminal amino acid residues, which are not present in P2, are critical for transposase activity. P2, in the absence of P1 or in the presence of a mutant P1, is not sufficient to mediate transposition. It is not known whether P2 has any function in the transposition reaction.

P2 is sufficient, however, to inhibit transposition. All mutants that perturb the synthesis or coding sequence of P1 but leave P2 unaltered are capable of inhibiting transposition as efficiently as the wild-type IS50R (this paper; Isberg et al., 1982; Johnson et al., 1982; Johnson & Reznikoff, 1984). The mechanism by which P2 inhibits transposition is not known but presumably it is due to a competitive interaction with P1. Since P2 shares the majority of the coding sequence of P1, it is not unreasonable to expect it to contain one or more of the activities of P1. Common functions for which P2 could compete with P1 include: binding to the ends (target sites) of the transposon, interaction with limiting host factors involved in transposition, or oligomerization leading to inactivation of a transposase complex by incorporation of P2 monomer(s).

The transposition frequency of Tn5 mutants that overproduce P2 relative to P1 was also investigated. Only when very high amounts of P2 were synthesized (i.e., an estimated 60 monomers of P2 to one monomer of P1) did significant repression of transposition occur. Doubling the synthesis of P2 had no measurable effect and increasing the synthesis of P2 approximately six times over that of the wild-type only inhibited transposition about twofold. These results demonstrated that P2 can inhibit the transposition of the element from which it was synthesized. However, the apparent inefficient repression of transposition by P2 suggests that: (1) multiple copies of P2 must be present per Tn5 molecule to effect repression; and (2) the repressing activity of this protein may not be the only factor that limits wild-type Tn5 transposition. This latter supposition is supported by the properties of a Tn5 mutant that decreases P2 synthesis relative to P1. This mutant transpose at a normal frequency (J. Yin & W. Reznikoff, unpublished results).

The experiments in this paper also suggest that the level of IS50R protein synthesis is not limiting Tn5 transposition. Varying the rate of functional IS50R protein synthesis per element over a 30-fold range has little effect on the transposition of Tn5 from a multi-copy plasmid to λ (Tables 5 and 7). This apparent lack of gene dosage dependence with transposition contrasts with Tn3, Tn10 and Tn903, whose transposition rate is dependent on the synthesis rate of the transposase (Chou et al., 1979; Gill et al., 1979; Morisato et al., 1983; N. Grindley, personal communication).

The experiments discussed above argue that the transposition frequency for wild-type Tn5 in E. coli as measured under steady-state conditions is not limited by the amount of P1 and P2 synthesized nor by the inhibitory activity of P2.
What then is controlling this rate? One possibility is that a host function, which participates in the transposition reaction, is limiting the frequency of the event. The presence of a host factor whose function may be required for Tn5 transposition is suggested by the analysis of the sequences at the termini of Tn5 that are required for transposition. Within the necessary terminal 18 bp a 9 bp sequence that is homologous to a repeated sequence found in the oriC region of a number of Gram-negative bacteria including E. coli (Johnson & Reznikoff, 1983). This sequence suggests that Tn5 may utilize some host replication functions during the transposition reaction, although we do not have any direct evidence for this. Such host functions, which are responsible for the initiation of chromosomal replication, may be present in relatively low quantities or could be utilized inefficiently in transposition and thus could be limiting the transposition rate of Tn5.

The authors thank J. Yin and Yong-di Zhang for permission to quote their unpublished data. This work was supported by grants from the National Science Foundation (PCM 8215021) and the National Institutes of Health (GM 19670).

REFERENCES

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*Note added in proof:* Recent experimental data (M. Krebs, R. Johnson & W. Reznikoff, unpublished results) suggests that the N terminus of protein 1 is encoded by the ATG at position 93 to 95 and not the GTG at 138 to 140. Thus protein 1 is 55 (not 40) amino acids longer than protein 2 and the mRNA which encodes protein 1 is not the transcript which starts at position 98.