Precise excision and instability of the transposon Tn5 in *Pseudomonas aeruginosa*

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Excision of the transposon Tn5 from sites of insertion in plasmid DNA was shown to occur at high frequency in *Pseudomonas aeruginosa*. Plasmids with Tn5 insertions conjugated poorly into *P. aeruginosa* and adversely affected growth compared to the respective parental plasmids. The kanamycin-resistance phenotype of Tn5 was expressed poorly in *P. aeruginosa* and kanamycin-sensitive strains were common during the manipulation of the *P. aeruginosa* transconjugants. Examination of plasmid DNA isolated from kanamycin-sensitive *P. aeruginosa* transconjugants revealed excision of Tn5 sequences. A plasmid containing a selectable marker (mercury resistance) inactivated by a Tn5 insertion was constructed, and Tn5 excised precisely, permitting the expression of the mercury-resistance marker at high frequency in *P. aeruginosa* and at the expected low frequency (10⁻¹) in *Escherichia coli*. The recombinational mechanism that promotes frequent Tn5 excision in *P. aeruginosa* operated in the absence of the *P. aeruginosa recA* gene product. Fragments of Tn5 were also examined for excision and instability in *P. aeruginosa*. A plasmid containing the terminal 485 bp of inverted repeat sequences from Tn5, but lacking the transposase or kanamycin-resistance genes, also showed precise excision of Tn5 DNA at high frequency (10⁻¹) in *P. aeruginosa*. Unlike plasmids containing a complete Tn5 insertion, this plasmid transferred to *P. aeruginosa* at normal frequencies and growth of the host was not severely impaired. In contrast, plasmids containing either IS50 element transferred to *P. aeruginosa* at greatly reduced frequencies, and transconjugants containing the IS50R element (which contains the active transposase gene) were small and especially difficult to maintain. *P. aeruginosa* transconjugants harbouring a plasmid containing only the DNA between the IS50 elements (which included the kanamycin-resistance gene) were of normal size and stably maintained. These observations suggest that frequent and precise excision of Tn5 in *P. aeruginosa* required the long inverted repeat sequences at the termini of Tn5. The adverse effects conferred by IS50 on the transconjugant formation and growth of *P. aeruginosa* were apparently not required to promote Tn5 excision.

Introduction

Transposable elements have served as some of the most powerful experimental tools available to the bacterial geneticist (Kleckner, 1981). They are often used to physically map genes of interest on recombinant plasmids because the sites of insertions can be readily determined by restriction analysis. For example, we have used the transposons Tn501 and Tn1 to map the location of genes on recombinant plasmids containing DNA fragments from *Pseudomonas aeruginosa* (Ohman et al., 1985; Goldberg & Ohman, 1987; Flynn & Ohman, 1988a; Horn & Ohman, 1988). Unfortunately, the target sites of Tn501 and Tn1 are typically non-random, and regions of DNA on recombinant plasmids can be difficult to analyse by transposon mutagenesis when only these two transposons are employed.

The transposon Tn5 has been used extensively as a tool for mutagenesis in many enteric and non-enteric Gram-negative species (Berg & Berg, 1983). It is one of the best-characterized transposons and demonstrates a nearly random choice of target sites (Berg, 1977). However, the use of Tn5 in *P. aeruginosa* has shown only limited success. When Stapleton *et al.* (1984) screened for Tn5-induced mutants of *P. aeruginosa* that were deficient in the production of extracellular proteases, a mutant obtained reverted at high frequency when the selection
pressure for the transposon-encoded kanamycin resistance was removed. O'Hoy & Krishnapillai (1985) isolated Tn5-induced auxotrophs, but compared to auxotrophs generated with Tn2521 (a native P. aeruginosa transposon), the frequency of stable mutations with pressure for the transposon-encoded kanamycin resistance for the transposon-encoded kanamycin resistance was considerably reduced and these mutants reverted to the parental genotype at a much higher frequency. Schilf & Krishnapillai (1985) reported a Tn5-containing plasmid which caused its P. aeruginosa host to form very small colonies. We and others have previously noted the general instability of Tn5 in Pseudomonas species (Goldberg & Ohman, 1987; Meyer et al., 1982).

In this study, we transferred recombinant plasmids containing Tn5 to P. aeruginosa to test for complementation of chromosomal mutations in order to physically map genes of interest, and we noted that the growth of the transconjugants was adversely affected by Tn5. Also, Tn5 sequences frequently excised from the plasmid DNA in P. aeruginosa even though another transposon (Tn501) did not demonstrate such instability under the same conditions. This behaviour of Tn5 in P. aeruginosa precluded our use of Tn5 for physical mapping of genes on recombinant plasmids in this species. We thus investigated the nature of Tn5 instability in order to improve our understanding of how transposable elements are maintained and lost in Pseudomonas.

Methods

Growth media. L medium, NY medium (Goldberg & Ohman, 1984), and minimal salts medium (MM) (Ohman & Chakrabarty, 1982) have been previously described. Media were solidified with 1.5% agar (Difco). Antibiotics were used in selection media at the following concentrations (per ml) unless specified otherwise: chloramphenicol (Cm), 30 μg for E. coli, test tubes, and plates, 10 μg for E. coli and 100 μg for P. aeruginosa; kanamycin (Km), 25 μg for E. coli and 350 μg for P. aeruginosa; mercuric chloride (Hg), 15 μg for both E. coli and P. aeruginosa. Hg-containing media were prepared fresh daily.

DNA techniques. Methods for the isolation of plasmid DNA from E. coli (Birnboim & Doly, 1979) and P. aeruginosa (Goldberg & Ohman, 1984) have been described. E. coli HB101 (Boyer & Roulland-Dussiau, 1969) was made competent for plasmid transformation by treatment with calcium chloride (Cohen et al., 1972). Restriction endonucleases (Boehringer-Manheim Biochemicals) and T4 DNA ligase (New England Biolabs) were used in enzyme reactions as specified by the manufacturer. DNA restriction fragments were examined using agarose gel electrophoresis with 0.7% agarose (FMC BioProducts) in 40 mM-Tris/acetate (pH 8.0), 1 mM-EDTA buffer.

Transposon insertion mutagenesis, plasmid construction, and mobilization of plasmids to P. aeruginosa. Two methods were employed to obtain Tn5 insertions in recombinant plasmids. A suicide plasmid pJG1 (conferring Tc resistance), and two EcoRI fragments (2.6 and 1.77 kb) from P. aeruginosa FRD1 that complement the algB50 mutation in FRD261. pJG1: Tn5-34 was obtained after transposition of Tn5 to P. aeruginosa sequences on pJG1. (Right) pHG1 consists of pLAFR1 and two EcoRI fragments (2.3 to 5.9 kb) from Tn501 that encode Hg'. pHG1: Tn5 was obtained after transposition of Tn5 to pHG1 followed by selection and screening for Km', Tc' and Hg'. Restriction endonuclease cleavage sites EcoRI (R) and HindIII (H) in pJG1 and pHG1 are shown.

Fig. 1. (Left) pJG1 has been previously described (Goldberg & Ohman, 1984) and consists of the broad-host-range plasmid pLAFR1 (conferring Tc') and two EcoRI fragments (2.6 and 1.77 kb) from P. aeruginosa FRD1 that complement the algB50 mutation in FRD261. pJG1: Tn5-34 was obtained after transposition of Tn5 to P. aeruginosa sequences on pJG1. (Right) pHG1 consists of pLAFR1 and two EcoRI fragments (2.3 to 5.9 kb) from Tn501 that encode Hg'. pHG1: Tn5 was obtained after transposition of Tn5 to pHG1 followed by selection and screening for Km', Tc' and Hg'. Restriction endonuclease cleavage sites EcoRI (R) and HindIII (H) in pJG1 and pHG1 are shown.

The Tn5 sequences in pHG1: Tn5-1 were modified as follows. To construct pH2 (Fig. 2), a chloramphenicol acetyltransferase (cat) cartridge (SalI fragment) from pCM1 (Close & Rodriguez, 1982) was ligated into the Xhol sites within the IS030 elements of pHG1: Tn5 and transformed into HB101; resultant Tc' transformants appeared Km' and Cm'. We could not construct a plasmid with the two 485 bp inverted repeats ligated together. To construct pH3 (Fig. 2), a cat cartridge (RamH1 fragment) from pCM4 (Close & Rodriguez, 1982) was ligated into the single BamHI site of pHG1: Tn5-1 (located in Tn5) and transformed into HB101; resultant Tc' transformants appeared Km' and slightly Cm'.
3428 bp HindIII fragment containing the Km' gene of Tn5 from pRZ102 (i.e. ColEl : Tn5) (Jorgensen et al., 1979) was ligated into the HindIII site of the broad-host-range plasmid pLAFR3 (Staskawicz et al., 1987) and transformed into HB101. To construct pJW10 and pJW11, which contained the left and right halves of Tn5, respectively, the EcoRI-BamHI fragments containing either the left or the right halves of Tn5 from pHG1 : Tn5-1 were ligated into the EcoRI-BamHI sites of pBR322 (Bolivar et al., 1977) and transformed into HB101. These plasmids were linearized with EcoRI, ligated into the EcoRI site of the broad-host-range plasmid pLAFR1 (Friedman et al., 1982), and transformed into HB101.

The conjugative helper plasmid pRK2013 (Figurski & Helinski, 1979) was used in tripertential matings to transfer recombinant plasmids to P. aeruginosa strains FRD1 (Ohman & Chakrabarty, 1981), FRD261 algB50 (Goldberg & Ohman, 1984), FRD284 recA7 (Ohman et al., 1985), PAO1 (Holloway et al., 1979), and PDO3 recA7 (Horn & Ohman, 1988). Samples (0.2 ml) of stationary-phase L broth cultures of E. coli HB101 (containing a recombinant plasmid), HB101(pRK23), and P. aeruginosa were mixed, collected on a membrane filter (25 mm diam., 0.45 µm pore size; Millipore), and incubated cell side up on the surface of an L agar plate for 6 to 24 h at 37 °C. Cells were resuspended in saline (5 ml) and spread onto MM agar supplemented with the appropriate antibiotics. Alginate production was assayed by incubating cell side up on the surface of an L agar plate for 6 to 24 h at 37 °C. Cells were resuspended in saline (5 ml) and spread onto MM agar supplemented with the appropriate antibiotics and tetracycline to select for transconjugants of the P. aeruginosa recipient. Following incubation at 37 °C for 2 d, transconjugant colonies were semi-purified on the same MM selective agar. Antibiotic resistance markers were scored by patching into NY agar containing the appropriate antibiotic. Alginate production was scored as positive by the appearance of a mucoid phenotype on L agar (Goldberg & Ohman, 1984).

Results

Reversion of algB :: Tn5 alleles in P. aeruginosa

pJG1 is a recombinant plasmid containing algB, a gene involved in the control of alginate production in P. aeruginosa (Goldberg & Ohman, 1984). The algB gene has been previously mapped on pJG1 using Tn501 mutagenesis and complementation analyses in the algB mutant strain P. aeruginosa FRD261 (Goldberg & Ohman, 1987). pJG1 was employed to test the use of Tn5 for physical mapping of genes from P. aeruginosa. Random Tn5 mutagenesis of pJG1 was performed, and the sites of Tn5 insertions in pJG1 were found to be generally random as expected (data not shown). The pJG1 :: Tn5 plasmids were transferred via tripertential mating to P. aeruginosa FRD261 with selection for Tc to test for complementation of the algB50 mutation. However, all of the FRD261(pJG1 :: Tn5) transconjugant colonies obtained grew poorly compared to FRD261(pJG1) transconjugants. Also, all plasmids were able to complement the defect for alginate production conferred by algB50 even though some of them had Tn5 insertions that mapped within the algB gene. pJG1 :: Tn5-34 (Fig. 1) was one plasmid that contained an algB :: Tn5 allele. FRD261(pJG1 :: Tn5-34) transconjugant colonies frequently (> 30%) did not demonstrate the Km' marker encoded by Tn5 when patched onto NY-Km agar. When Km' FRD261(pJG1 :: Tn5-34) strains were passaged once on NY-Tc agar (to maintain selection for the vector), most of the resultant colonies appeared Km' and produced alginate, which suggested that precise excision of Tn5 from algB had occurred. Similar loss of Km' was observed when pJG1 :: Tn5 plasmids were transferred to P. aeruginosa strains FRD1 (wild-type) and PAO1 (a common laboratory strain), and to recA mutants FRD284 and PDO3 (a recA mutant of PAO).

Further examination of the behaviour of Tn5 in P. aeruginosa was continued in strain PAO1. As in strain FRD, PAO1(pJG1 :: Tn5-34) transconjugant colonies were small compared to PAO1(pJG1), and > 30% of the PAO1(pJG1 :: Tn5-34) transconjugants appeared Km'. Km' strains of PAO1(pJG1 :: Tn5-34) were taken from Tc-medium, rather than from Km-medium, to avoid the possibility of spontaneous Km' (which is common in P. aeruginosa (Bryan et al., 1980)), and were grown in L broth with Tc (50 µg ml⁻¹) overnight. Following subculture, 60 to 100% of the colonies obtained again appeared Km' or showed poor growth on Km. The PAO1(pJG1) control cultures treated in the same fashion showed no growth on Km plates.

Plasmids isolated from Km' PAO1(pJG1 :: Tn5-34) transconjugants (20 examined) were transformed into E. coli (with selection for Tc') and shown to have restriction fragment profiles identical to the parental plasmid pJG1, which indicated that the Tn5 sequences had excised in P. aeruginosa (Fig. 3). Plasmids isolated from PAO1(pJG1 :: Tn5-34) strains that demonstrated poor growth on Km were transformed into E. coli (with selection for Tc'), and approximately half of these transformants were Km' and contained either pJG1-like plasmids (indicating Tn5 excision) or showed various deletions of DNA from the recombinant plasmid (data not shown). The Km' phenotype conferred by pJG1 :: Tn5-34 was stable in E. coli as expected despite its behaviour in P. aeruginosa.

Tn5 excises precisely at high frequency in P. aeruginosa

To measure the frequency of precise Tn5 excision in P. aeruginosa, plasmid pHG1 :: Tn5-1 (Fig. 1) was constructed (see Methods) that had a Hg' determinant which was insertionally inactivated by Tn5. Precise excision of Tn5 from sites of insertion has been reported to occur in E. coli at a frequency of approximately 10⁻⁴ per cell (Berg, 1977). When E. coli HB101(pHG1 :: Tn5-1) was plated on NY-Hg agar, Hg' colonies were observed at a frequency of approximately 10⁻⁴ to 10⁻⁵ per cell plated (Fig. 2). Thus, the frequency of Tn5 excision in E. coli
had not been significantly altered due to some consequence of its construction.

Similar to the results obtained above with pJG1::Tn5-34, PAO1(pHG1::Tn5-1) transconjugant colonies were small compared to PAO1(pHG1) and were scored as Km\(^\text{r}\) (> 30\%) or slow growing in the presence of Km. When tested on Hg medium, approximately 1% of the PAO1(pHG1::Tn5-1) transconjugant colonies (selected on Tc-media) had regained the Hg\(^\text{r}\) marker and were always Km\(^\text{r}\), thus indicating precise excision of Tn5. When cultures of Km\(^\text{r}\) PAO1(pHG1::Tn5) transconjugants were plated directly onto NY-Hg and NY-Tc agar, Hg\(^\text{r}\) colonies appeared to be identical to pHG1, ligated into the HindIII (H) and BamHI (B) are shown. Vector transfer frequency indicates the formation of transconjugant colonies with these plasmids in PAO1 compared to the number obtained with the vector pLAFR1. Slow growth of P. aeruginosa transconjugants indicates that very small unstable colonies were obtained. Restriction endonuclease cleavage sites for XhoI

**Fig. 2.** Map of Tn5 and its derivatives in broad-host-range plasmids. pHG1::Tn5-1 contains Tn5 within the Hg\(^\text{r}\) gene of Tn501. pHG2 contains a SalI cat cartridge (Close & Rodriguez, 1982) ligated into the XhoI sites in the inverted repeats of pHG1::Tn5-1. pHG3 contains a BamHI cat cartridge (Close & Rodriguez, 1982) ligated into a single central BamHI site of pHG1::Tn5-1. pLAKM1 contains the HindIII fragment of pRZ102 (Jorgensen et al., 1979), including the Km\(^\text{r}\) gene, ligated into the HindIII site of pLAFR3 (Staskawicz et al., 1987). pJW10 and pJW11 contain the left and right EcoRI-BamHI fragments of pHG1::Tn5-1, respectively, ligated into the EcoRI-BamHI sites of pBR322 (Bolivat et al., 1977) and then linearized with EcoRI and cloned into pLAFR1 (Friedman et al., 1982).

Spontaneous precise excision was measured after transferring plasmids to E. coli or P. aeruginosa with the initial selection for Tc\(^\text{r}\) and Hg\(^\text{r}\). Vector transfer frequency indicates the formation of transconjugant colonies with these plasmids in PAO1 compared to the number obtained with the vector pLAFR1. Slow growth of P. aeruginosa transconjugants indicates that very small unstable colonies were obtained. Restriction endonuclease cleavage sites for XhoI

Effect of Tn5 deletion derivatives on P. aeruginosa growth

Like PAO1(pHG1::Tn5-1), PAO1(pHG3) transconjugants showed reduced growth and were scored as Km\(^\text{r}\) or slow growing in the presence of Km. However, PAO1(pHG2) transconjugant colonies showed normal

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<th>Tn5 in plasmid</th>
<th>IS50L</th>
<th>IS50R</th>
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<tr>
<td>pHG1::Tn5-1</td>
<td>X</td>
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<td>pJW11</td>
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Spontaneous precise excision

- **E. coli**
  - pHG1::Tn5-1: 10\(^{-7}\)
  - pHG2: 10\(^{-6}\)
  - pHG3: 10\(^{-7}\)
  - pLAKM1: ND
  - pJW10: ND
  - pJW11: ND

- **P. aeruginosa**
  - pHG1::Tn5-1: 10\(^{-3}\)
  - pHG2: 10\(^{-2}\)
  - pHG3: 10\(^{-3}\)
  - pLAKM1: ND
  - pJW10: ND
  - pJW11: ND

Vector transfer frequency (%)

- pHG1::Tn5-1: 85
- pHG2: 95
- pHG3: 95
- pLAKM1: 90
- pJW10: 10
- pJW11: 10

Growth of P. aeruginosa transconjugants

- Slow
- Normal
Excision of Tn5 in Pseudomonas aeruginosa

Fig. 3. Physical evidence for Tn5 excision from plasmid DNA following residence in P. aeruginosa. Shown is an agarose gel containing the products of EcoRI digestions of the following plasmids isolated from E. coli. Lanes: 1, pJG1::Tn5-34; 2, pJG1; 3 and 4, pJG1::Tn5-34 plasmids which were Km' following replication in P. aeruginosa and transformation into E. coli; 5, pHG1::Tn5; 6, pHG1; 7, pHG1::Tn5-1 which was weakly Km' and Hg' following replication in P. aeruginosa and appeared Km' Hg' after transformation into E. coli; 8, pHG1::Tn5 which was Km' Hg' following replication in P. aeruginosa and transformation into E. coli.

growth. This indicated that the Tn5 sequences deleted in pHG2 were responsible for the adverse effect on growth in P. aeruginosa but were apparently not required to promote frequent Tn5 excision. To determine which portions of Tn5 led to the small colony morphology and poor maintenance in P. aeruginosa of plasmids carrying Tn5, we constructed (see Methods) plasmids with deletion derivatives of Tn5 (Fig. 2). pLAKM1 contained the central 3428 bp HindIII fragment from Tn5 (including the Km' gene). pJW10 contained the left half of Tn5 including the IS50L element (which encodes an inactive transposase) and the Km' gene. pJW11 contained the right half of Tn5 including the IS50R element (which encodes the active transposase gene and a transposition-inhibitor gene). Following selection for the vector-encoded Tc', PAO1(pLAKM1) transconjugant colonies were of normal size, formed at normal frequencies (i.e. about 90% when vector alone was transferred), and appeared stably Tc' and Km'. However, both pJW10 and pJW11 transferred inefficiently to PAO1, with transconjugants forming at frequencies of 1 to 10% of those obtained with vector pLAFR1. PAO1(pJW10) colonies appeared normal once obtained, and they could be maintained on NY-Tc agar. However, PAO1(pJW11) colonies appeared very small and were difficult to maintain on NY-Tc agar. PAO1(pJW11) readily lost the plasmid when selection for Tc' was removed. Thus, IS50R was responsible for the difficulty in maintenance of Tn5-containing plasmids in P. aeruginosa.

Discussion

In this study, we investigated the basis for the marked instability of Tn5 on plasmids in P. aeruginosa. This instability included difficulty in plasmid maintenance, and excision of Tn5 sequences, two phenotypes which we found were independent. When plasmids containing Tn5 were conjugally transferred to P. aeruginosa, transconjugants grew poorly, indicating that Tn5 contained sequences that have a detrimental effect on either plasmid maintenance and/or cell viability in P. aeruginosa. This effect was not observed with plasmids containing either the DNA between the two IS50 elements of Tn5 (including the Km' gene) or the terminal 485 bp of the transposon. The two IS elements of Tn5, IS50L and IS50R, differ only in a single base pair (Rothstein & Reznikoff, 1981). IS50R contains both the transposase gene and the overlapping transposition-inhibitor gene, which utilizes a promoter downstream of the transposase promoter (Johnson et al., 1982; Isberg et al., 1982). The IS50L element has an ochre mutation in these two genes (Rothstein & Reznikoff, 1981) and encodes inactive truncated proteins (Johnson et al., 1982). Plasmids containing either IS50 element of Tn5 were transferred inefficiently to P. aeruginosa, and a plasmid containing the IS50R (encoding the active transposase) formed small colonies of P. aeruginosa and was especially unstable. The Tn5 transposase may be overexpressed and thus detrimental to P. aeruginosa, or the transposition-inhibitor gene product may be inefficiently produced and unable to control transposase gene expression in P. aeruginosa. P. aeruginosa is known to utilize different promoters than E. coli (Deretic et al.,
1987). Others have transposed Tn5 in \textit{P. aeruginosa} (Stapleton et al., 1984; Sokol, 1987), which indicates that the transposase gene is expressed in this species. The transposase gene product of IS50L is defective for transposition, and we observed that \textit{P. aeruginosa} colonies containing plasmids with IS50L were stable whereas those containing IS50R were unstable.

The excision of Tn5 has been observed in such diverse micro-organisms as the cyanobacterium \textit{Anacystis nidulans} R2 (Gendel, 1987) and the yeast \textit{Saccharomyces cerevisiae} (Gordenin et al., 1988). In \textit{A. nidulans} the degree of instability was dependent upon the location of the insert on the plasmid DNA (Gendel, 1987). In \textit{S. cerevisiae}, the frequency of Tn5 excision (approximately $10^{-5}$) was about the same as or lower than that found in \textit{E. coli} (approximately $10^{-6}$) (Gordenin et al., 1988). In \textit{P. aeruginosa}, we found that excision of Tn5 was independent of its site of insertion and occurred at frequencies four orders of magnitude higher than in \textit{E. coli}. When plasmids containing Tn5 insertions were transferred to \textit{P. aeruginosa}, transconjugants often contained plasmids that appeared identical to the parental plasmid, indicating excision of Tn5 from the plasmid DNA. Transposition of Tn5 to other sites in the genome was never observed following Tn5 excision. Using a plasmid with a Tn5 insertion inactivating a Hgr selectable marker, precise excision was shown to occur at high frequency ($10^{-2}$ to $10^{-3}$) in \textit{P. aeruginosa} even though it occurred at the expected low frequency ($10^{-6}$ to $10^{-7}$) in \textit{E. coli}. This phenomenon of frequent and spontaneous Tn5 excision was observed in two different strains of \textit{P. aeruginosa} (FRD and PAO) as well as in \textit{recA} mutants of these strains. Thus, spontaneous excision of Tn5 occurred in \textit{P. aeruginosa} by some recombinational mechanism that does not require a \textit{recA}-dependent pathway.

Tn5 is a composite transposon containing two IS50 elements of 1532 bp in inverted orientation that flank a 2754 bp region containing a Km' gene. Excision of Tn5 in \textit{E. coli} has been studied extensively and occurs at a frequency of approximately $10^{-6}$ (Berg, 1977). The excision event is distinct from the transposition event in that it does not involve the transposon-encoded transposase. Excision of Tn5 from sites of insertion is dependent on the terminal inverted sequences that result from the inverted orientation of the two IS50 elements; excision is inefficient when the IS50 elements are placed in direct rather than inverted orientation (Egner & Berg, 1981). It was postulated that any DNA segment containing long terminal inverted repeats may be able to excise by a mechanism of copy error following denaturation and intramolecular reannealing (Berg et al., 1980). The smallest inverted repeats that had been tested in previous studies for precise excision were 1195 bp in length (Berg et al., 1980; Egner & Berg, 1981). In this study, we found that 485 bp of inverted repeats of Tn5 were sufficient to allow precise excision from plasmids in both \textit{E. coli} and \textit{P. aeruginosa}. It is interesting that the precise excision seen with this plasmid (pHG2) in both \textit{E. coli} and \textit{P. aeruginosa} is consistently ten times more frequent than with Tn5 alone. The reason for this is unknown, but may reflect the smaller size of the insert between the ends of Tn5 bringing the inverted repeats closer together.

Egner & Berg (1981) proposed a basic model for Tn5 excision which may also operate in \textit{P. aeruginosa} (Fig. 4). When double-stranded DNA is denatured and rendered single-stranded (e.g. during DNA replication), complementary sequences of the inverted IS50 elements can form intramolecular double-stranded segments. The preferential use of single-stranded DNA as a template in DNA synthesis favours the slippage of the end of the nascent chain between the first and second copy of the 9 base repeat, continuation of synthesis, and excision of Tn5 following continuation of DNA synthesis. Although this deletion process is rare ($10^{-6}$ to $10^{-7}$), but normal, in \textit{E. coli}, it occurs at high frequency ($10^{-2}$ to $10^{-3}$) in \textit{P. aeruginosa}.

\begin{figure}[h]
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\caption{Model for Tn5 excision according to Egner & Berg (1981) which may also operate in \textit{P. aeruginosa}. If double-stranded DNA is denatured and rendered single-stranded, such as during DNA replication, complementary sequences of the inverted IS50 elements can form intramolecular double-stranded segments. The preferential use of single-stranded DNA as a template in DNA synthesis favours the slippage of the end of the nascent chain between the first and second copy of the 9 base repeat, continuation of synthesis, and excision of Tn5 following continuation of DNA synthesis. Although this deletion process is rare ($10^{-6}$ to $10^{-7}$), but normal, in \textit{E. coli}, it occurs at high frequency ($10^{-2}$ to $10^{-3}$) in \textit{P. aeruginosa}.}
\end{figure}


is rare, but normal, in E. coli, it occurs at high frequency in P. aeruginosa.

Excision of DNA containing long inverted repeat sequences may be an adaptation by P. aeruginosa to eliminate composite transposons like Tn5 that attempt to parasitize its genome. Although transposons like Tn1 and Tn501 (characterized by short terminal inverted repeat sequences) are often found in P. aeruginosa, we are unaware of any composite transposons (characterized by long terminal inverted repeat sequences) being demonstrated in this species. Although the requirements for excision of Tn5 in P. aeruginosa appear to be similar to those in E. coli, the frequency is much higher due to some enhancement inherent in P. aeruginosa. It is interesting to note that mutations have been found in E. coli that stimulate excision of Tn5 and Tn10 which are unusual alleles of recBC; such mutations probably increase denaturation of DNA (Lundblad et al., 1984). P. aeruginosa may contain a recBC analogue which encodes a product that promotes DNA denaturation and may promote Tn5 deletion. Genes analogous to recBC have not yet been identified in P. aeruginosa. When we used the cloned E. coli recBC gene as a probe [kindly provided by S. Kushner (Dykstra, et al., 1984)] in Southern blot hybridization, no homology to P. aeruginosa chromosomal DNA was detected even under relaxed conditions (this laboratory, unpublished data). This indicates that the recBC genes of these two bacteria are significantly divergent at the DNA level. It is possible that the wild-type P. aeruginosa recBC allele plays a significant role in how transposable elements are maintained and lost in this organism. This may also be reflected in the different recombinational and DNA repair phenotypes of these two bacteria (Ohman et al., 1985), one result of which may be the high frequency of excision of the transposon Tn5 observed in P. aeruginosa.

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