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 (10^{-3}) in *P. aeruginosa* and at the expected low frequency (10^{-7}) 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^{-2}) 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

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pressure for the transposon-encoded kanamycin resistance was removed. O'Hoy & Krishnapillai (1985) isolated Tn5-induced auxotrophs, but compared to auxotrophs generated with Tn5221 (a native *P. aeruginosa* transposon), the frequency of stable mutations with Tn5 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). We have reported a gene replacement technique in *P. aeruginosa*, called excision marker rescue, which takes advantage of Tn5 instability to promote the exchange of plasmid-borne alleles for homologous chromosomal sequences (Goldberg & Ohman, 1987; Flynn & Ohman, 1988b). Others have also used this technique (Woodruff & Hancock, 1988).

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*; tetracycline (Tc), 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-Dussoix, 1969) was made competent for plasmid transformation by treatment with calcium chloride (Cohen *et al.*, 1972). Restriction endonucleases (Boehringer-Mannheim 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.

Fig. 1. (Left) pJG1 has been previously described (Goldberg & Ohman, 1984) and consists of the broad-host-range plasmid pLAFR1 (confering Tc^r) and two *Eco*RI fragments (2.6 and 17.7 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 *Eco*RI fragments (2.3 to 5.9 kb) from Tn501 that encode Hg^r. pHG1::Tn5 was obtained after transposition of Tn5 to pHG1 followed by selection and screening for Km^r, Tc^r and Hg^r. Restriction endonuclease cleavage sites *Eco*RI (R) and *Hind*III (H) in pJG1 and pHG1 are shown.

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 λ ::Tn5 (Berg, 1977) was used to introduce Tn5 into *E. coli* HB101 containing a target plasmid. Kanamycin-resistant colonies were pooled, and the resultant plasmids were isolated and transformed into *E. coli* HB101 with selection for kanamycin resistance (Km^r). In other experiments, *E. coli* containing a target plasmid (which included a λ cos site) and plasmid RSF1010::Tn5 (as the donor of Tn5) was used as the host for the preparation of a λ cl857 lysate and *in vivo* packaging of the cosmids (Ohman *et al.*, 1985; Ohman, 1986). The lysate was then used to transduce Tn5-containing target plasmids into *E. coli* HB101 by selection for Km^r.

pJG1 contains a 20.3 kb fragment of DNA from alginate-producing *P. aeruginosa* strain FRD1 in the tetracycline resistant (Tc^r) broad-host-range cosmid vector pLAFR1 (Friedman *et al.*, 1982). pJG1 complements an alginate mutation *algB50* in the mutant FRD261 (Goldberg & Ohman, 1984). pJG1::Tn5-34 (Fig. 1) was obtained using the *in vivo* packaging method for Tn5 mutagenesis as described above.

pHG1 was derived from pJG1::Tn501-124 (Goldberg & Ohman, 1987) by partial digestion with *Eco*RI, treatment with T4 DNA ligase, and transformation into *E. coli* HB101 with selection for Tc^r and mercury resistance (Hg^r). pHG1 retained the two internal *Eco*RI fragments (2.3 and 5.9 kb) of Tn501 which encoded Hg^r. pHG1::Tn5-1 (Fig. 1) was derived from pHG1 using λ ::Tn5 as described above to obtain this Hg^r derivative with a Tn5 insertion located within the DNA sequences encoding Hg^r (Misra *et al.*, 1984).

The Tn5 sequences in pHG1::Tn5-1 were modified as follows. To construct pHG2 (Fig. 2), a chloramphenicol acetyltransferase (*cat*) cartridge (*Sal*I fragment) from pCM1 (Close & Rodriguez, 1982) was ligated into the *Xho*I sites within the IS50 elements of pHG1::Tn5 and transformed into HB101; resultant Tc^r transformants appeared Km^s and Cm^r. We could not construct a plasmid with the two 485 bp inverted repeats ligated together. To construct pHG3 (Fig. 2), a *cat* cartridge (*Bam*HI fragment) from pCM4 (Close & Rodriguez, 1982) was ligated into the single *Bam*HI site of pHG1::Tn5-1 (located in Tn5) and transformed into HB101; resultant Tc^r transformants appeared Km^r and slightly Cm^r. To construct pLAKM1 (Fig. 2), the

3428 bp *Hind*III fragment containing the *Km^r* gene of Tn5 from pRZ102 (i.e. ColE1::Tn5) (Jorgensen *et al.*, 1979) was ligated into the *Hind*III 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 *Eco*RI–*Bam*HI fragments containing either the left or the right halves of Tn5 from pHG1::Tn5-1 were ligated into the *Eco*RI–*Bam*HI sites of pBR322 (Bolivar *et al.*, 1977) and transformed into HB101. These plasmids were linearized with *Eco*RI, ligated into the *Eco*RI 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 triparental matings to transfer recombinant plasmids to *P. aeruginosa* strains FRD1 (Ohman & Chakrabarty, 1981), FRD261 *algB*50 (Goldberg & Ohman, 1984), FRD284 *recA*7 (Ohman *et al.*, 1985), PAO1 (Holloway *et al.*, 1979), and PDO3 *recA*7 (Horn & Ohman, 1988). Samples (0.2 ml) of stationary-phase L broth cultures of *E. coli* HB101 (containing a recombinant plasmid), HB101(pRK2013), 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 amino acids 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 onto 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 triparental mating to *P. aeruginosa* FRD261 with selection for Tc^r to test for complementation of the *algB*50 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 *algB*50 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^r* marker encoded by Tn5 when patched onto NY-Km agar. When *Km^r* 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^s* and produced alginate, which suggested that precise excision of Tn5 from *algB* had occurred. Similar loss of *Km^r* 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^s*. *Km^r* strains of PAO1(pJG1::Tn5-34) were taken from Tc-medium, rather than from Km-medium, to avoid the possibility of spontaneous *Km^r* [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^s* 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^s* PAO1(pJG1::Tn5-34) transconjugants (20 examined) were transformed into *E. coli* (with selection for Tc^r) 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^r), and approximately half of these transformants were *Km^s* and contained either pJG1-like plasmids (indicating Tn5 excision) or showed various deletions of DNA from the recombinant plasmid (data not shown). The *Km^r* 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^r 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 10⁻⁶ per cell (Berg, 1977). When *E. coli* HB101(pHG1::Tn5-1) was plated on NY-Hg agar, Hg^r colonies were observed at a frequency of approximately 10⁻⁶ to 10⁻⁷ per cell plated (Fig. 2). Thus, the frequency of Tn5 excision in *E. coli*

Fig. 2. Map of Tn5 and its derivatives in broad-host-range plasmids. pHG1::Tn5-1 contains Tn5 within the Hg^r gene of Tn501. pHG2 contains a *Sal*I *cat* cartridge (Close & Rodriguez, 1982) ligated into the *Xho*I sites in the inverted repeats of pHG1::Tn5-1. pHG3 contains a *Bam*HI *cat* cartridge (Close & Rodriguez, 1982) ligated into a single central *Bam*HI site of pHG1::Tn5-1. pLAKM1 contains the *Hind*III fragment of pRZ102 (Jorgensen *et al.*, 1979), including the Km^r gene, ligated into the *Hind*III site of pLAFR3 (Staskawicz *et al.*, 1987). pJW10 and pJW11 contain the left and right *Eco*RI–*Bam*HI fragments of pHG1::Tn5-1, respectively, ligated into the *Eco*RI–*Bam*HI sites of pBR322 (Bolivar *et al.*, 1977) and then linearized with *Eco*RI 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^r and Hg^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 *Xho*I (X), *Hind*III (H) and *Bam*HI (B) are shown. ND, Not determined.

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^s (> 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^r marker and were always Km^s, thus indicating precise excision of Tn5. When cultures of Km^r PAO1(pHG1::Tn5) transconjugants were plated directly onto NY-Hg and NY-Tc agar, Hg^r colonies appeared at frequencies ranging from 10⁻² to 10⁻³ per cell plated. Plasmids isolated from Hg^r strains of PAO1(pHG1::Tn5) and transformed into *E. coli* HB101 (by selection for Tc^r) demonstrated a restriction pattern identical to pHG1 (Fig. 3). The plasmids from weakly Km^r PAO1(pHG1::Tn5) strains transformed into *E. coli* HB101 (by selection for Tc^r) appeared to be identical to pHG1::Tn5 (Fig. 3).

To test whether the high frequency of Tn5 excision in *P. aeruginosa* was promoted by the intact IS50 elements, or by the DNA between the IS50 elements, a derivative of pHG1::Tn5 was constructed (see Methods) in which a chloramphenicol acetyltransferase (*cat*) gene cartridge replaced the central region of Tn5. This plasmid, pHG2

(Fig. 2), retained only 485 bp of inverted repeat sequences from the termini of the IS50 elements flanking the *cat* gene. The *cat* gene was also inserted in the middle of Tn5 (i.e. pHG3) to control for any effects on Tn5 excision conferred by *cat* (Fig. 2). When *E. coli* HB101(pHG2) and HB101(pHG3) were plated onto NY-Hg agar, Hg^r colonies were observed at the expected frequency of 10⁻⁶ to 10⁻⁷ per cell plated. Both PAO1(pHG2) and PAO1(pHG3) transconjugant colonies (selected for Tc^r) were found to have regained the Hg^r marker without selection at a frequency of about 1%, indicating precise excision of Tn5. These Hg^r colonies were always Km^s. When cultures of Hg^s PAO1(pHG2) and PAO1(pHG3) were plated directly onto NY-Hg and NY-Tc agar, Hg^r colonies appeared at frequencies ranging from 10⁻² to 10⁻³ per cell plated. Thus, only 485 bp (or less) of the long inverted repeat sequences of Tn5 were required to obtain frequent and spontaneous excision of Tn5 in *P. aeruginosa*.

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^s or slow growing in the presence of Km. However, PAO1(pHG2) transconjugant colonies showed normal

Fig. 3. Physical evidence for Tn5 excision from plasmid DNA following residence in *P. aeruginosa*. Shown is an agarose gel containing the products of *Eco*RI 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^s following replication in *P. aeruginosa* and transformation into *E. coli*; 5, pHG1::Tn5; 6, pHG1; 7, pHG1::Tn5-1 which was weakly Km^r and Hg^s following replication in *P. aeruginosa* and appeared Km^r Hg^s after transformation into *E. coli*; 8, pHG1::Tn5 which was Km^s Hg^r 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 *Hind*III fragment from Tn5 (including the Km^r gene). pJW10 contained the left half of Tn5

including the IS50L element (which encodes an inactive transposase) and the Km^r 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^r , 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^r and Km^r . 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^r 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^r 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 *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 *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 *Anacystis nidulans* R2 (Gendel, 1987) and the yeast *Saccharomyces cerevisiae* (Gordenin *et al.*, 1988). In *A. nidulans* the degree of instability was dependent upon the location of the insert on the plasmid DNA (Gendel, 1987). In *S. cerevisiae*, the frequency of Tn5 excision (approximately 10^{-8}) was about the same as or lower than that found in *E. coli* (approximately 10^{-6}) (Gordenin *et al.*, 1988). In *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 *E. coli*. When plasmids containing Tn5 insertions were transferred to *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 Hg^r selectable marker, precise excision was shown to occur at high frequency (10^{-2} to 10^{-3}) in *P. aeruginosa* even though it occurred at the expected low frequency (10^{-6} to 10^{-7}) in *E. coli*. This phenomenon of frequent and spontaneous Tn5 excision was observed in two different strains of *P. aeruginosa* (FRD and PAO) as well as in *recA* mutants of these strains. Thus, spontaneous excision of Tn5 occurred in *P. aeruginosa* by some recombinational mechanism that does not require a *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^r gene. Excision of Tn5 in *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

Fig. 4. Model for Tn5 excision according to Egner & Berg (1981) which may also operate in *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 *E. coli*, it occurs at high frequency (10^{-2} to 10^{-3}) in *P. aeruginosa*.

that 485 bp of inverted repeats of Tn5 were sufficient to allow precise excision from plasmids in both *E. coli* and *P. aeruginosa*. It is interesting that the precise excision seen with this plasmid (pHG2) in both *E. coli* and *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 *P. aeruginosa* (Fig. 4). When double-stranded DNA is denatured and rendered single-stranded (e.g. during DNA replication), complementary sequences such as those of the inverted IS50 elements can form double-stranded segments. The preferential use of single-stranded DNA as a template in DNA synthesis will favour 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 (Egner & Berg, 1981). Although this deletion process

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 be also 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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