Tn5tacl, a derivative of transposon Tn5 that generates conditional mutations

(Insertion sequence/Escherichia coli/plasmid pBR322/ lac repressor/isopropyl beta-d-thiogalactoside)

WEI-YUAN CHOW* and DOUGLAS E. BERG

Departments of Microbiology and Immunology and of Genetics, Campus Box 8093, Washington University Medical School, Saint Louis, MO 63110

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ABSTRACT

Conditional lethal mutations are valuable for analyzing essential genes. We describe here a derivative of the bacterial transposon Tn5 called Tn5tacl and its use in an innovative strategy for making mutations with conditional phenotypes. The 4.6-kilobase Tn5tacl element contains a strong, regulatable, outward-facing promoter (P$_{tac}$) near one end and is polar on the expression of distal genes when the inducer of P$_{tac}$ [isopropyl beta-d-thiogalactoside (IPTG)] is absent. Our results show that two unusual conditional mutant phenotypes result from Tn5tacl insertion in Escherichia coli: one is corrected by IPTG while the other is induced by IPTG. The broad host range of Tn5 and the conditional nature of these mutant phenotypes makes Tn5tacl well suited for identifying essential genes in diverse bacterial species.

Analyses of vital processes such as DNA replication, morphogenesis, and cell division in prokaryotes and eukaryotes have been greatly aided by mutations that confer conditional lethal phenotypes—nonsense mutations that cause translation to stop prematurely and are suppressed by specific mutant tRNAs and missense mutations that cause heat-, cold-, or osmotic pressure-sensitive phenotypes (1–8). The sets of genes identified in such studies depends, however, on which conditionally mutant phenotype is selected (4, 7, 8). With missense mutations this is primarily attributable to differences among proteins in the chance that an amino acid substitution will impair the protein’s function under the nonpermissive growth condition. The mutant yields are also biased by the distribution of codons that can be changed to nonsense triplets in one step and by the nonrandomness of spontaneous and induced mutation events. Because studies of vital functions in Escherichia coli have relied most heavily on temperature-sensitive mutations (5, 6), it is likely that many of the genes important in bacterial growth have not yet been identified.

We have sought more efficient ways of generating conditional mutations and describe here a strategy using a derivative of the bacterial transposon Tn5. During the last decade, resistance transposons have become valuable tools for molecular genetic research (9, 10). They can transpose to new sites without extensive DNA sequence homology in many organisms, generate null insertion mutations, and disrupt transcription distal to their sites of insertion. Although transpositions are rare, they can be obtained easily by selection for the element’s resistance trait. Despite the widespread use of these elements, they have not been exploited much in the search for essential genes. This is because their insertion generates absolute mutations that are lethal when in an essential gene, rather than mutations whose effect on phenotype is suppressed or reversed under permissive growth conditions.

The derivative of Tn5 described here, Tn5tacl (Fig. 1), was designed for the isolation of transposon insertion mutations that cause conditional phenotypes. It contains a P$_{tac}$ promoter near one end, placed so that it can elicit the transcription of adjacent target DNA, and also lacI, the gene whose product represses transcription from P$_{tac}$ unless an inducer [isopropyl beta-d-thiogalactoside (IPTG)] is present. A P$_{tac}$-containing derivative of Tn721 (which unlike Tn5 inserts preferentially into plasmid DNA) has been constructed by others (17). The present studies using plasmid and chromosomal DNA targets show that two unusual classes of conditional mutations are generated by Tn5tacl transposition in addition to standard insertion mutations. One class is corrected by IPTG and results from the failure to transcribe nearby genes. The other is sensitive to IPTG and results from excessive transcription of the nearby DNA segment.

MATERIALS AND METHODS

The bacterial strains used are derivatives of E. coli K-12 (Table 1). Bacteria were grown in LN (complex) medium or in M9 (minimal) glucose medium solidified with 1.5% agar as appropriate (13, 25). Antibiotics were used at the following concentrations: ampicillin, 250 μg/ml; kanamycin, 60 μg/ml; and tetracycline, 12 μg/ml (or 6 μg/ml where noted). IPTG was used at 0.5 mM. Standard procedures were used for bacterial growth, plasmid DNA preparation, restriction endonuclease digestion, electrophoresis, and transformation (13). DNA sequencing was carried out by a modification of the chain-termination method (12, 26). All enzymes were obtained from commercial suppliers (New England Biolabs, Boehringer Mannheim, or Bethesda Research Laboratories) and used according to the manufacturer’s recommendations.

The oligonucleotides used as sequencing primers are: A, 5’-GTATACAGGGGCTG (pBR322 positions 4334–4348); B, 5’-GCAATTTAAGCTGAT (pBR322 positions 64–49); C, 5’-GATAAGCTGTCAAC (pBR322 positions 22–8); and D, 5’-GCAAATTCGCGGATGC. Oligonucleotides A, B, and C were from New England Biolabs; oligonucleotide D was made on an Applied Biosystems 380A DNA synthesizer. Oligonucleotides A and B are specific for DNAs that flank Tn5tacl in the plasmid in which it was constructed and were used as primers in reactions that confirmed the sequences at the transposon ends. Oligonucleotides C and D match sequences within Tn5tacl near its ends (Fig. 1 legend). Oligonucleotide C also matches the HindIII–EcoRI segment of pBR322 and was used as a sequencing primer for inserts in pBR322 targets only after this segment was removed from the target DNA.

Abbreviations: IPTG, isopropyl beta-d-thiogalactoside; AmpR and TetR, ampicillin- and tetracycline-sensitive phenotypes; TetR and KanR, tetracycline- and kanamycin-resistant phenotypes; IS, insertion sequence.

*Present address: Institute of Molecular Biology, Academia Sinica, NanKang, Taipei 11529, Taiwan, Republic of China.
Transposition of Tn5sac1 from pBRG1410, the plasmid in which it was constructed, to phase λ b221 cl857 Oam29 Pam80, which is integration deficient and defective in replication in a nonsuppressing host, was obtained by growth of the plasmid on Sup+ cells carrying pBRG1410 and then transduction of the Nus− Sup+ strain DB509 to the kanamycin-resistant (KanR) phenotype. The nus mutation in DB509 interferes with the λ N gene function and causes infecting λ phase to form unstable plasmids. The few phase

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD1527</td>
<td>met hsdR supE supF rpsL</td>
<td>T. Kazic*</td>
</tr>
<tr>
<td>594</td>
<td>gal rpsL</td>
<td>Ref. 18</td>
</tr>
<tr>
<td>DB5015</td>
<td>W3110 trpEam9851, Eam9829 ΔtrpA229-1onB</td>
<td>Ref. 19</td>
</tr>
<tr>
<td>G13</td>
<td>nusA supE</td>
<td>S. Adhya†</td>
</tr>
<tr>
<td>JM101</td>
<td>F' lacI</td>
<td>J. Messing‡</td>
</tr>
<tr>
<td>MC1061</td>
<td>F− araD139Δ(ara-leu)7977 ΔlacX74 galU galK hsd hsm rpsL</td>
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<td>Mucts62</td>
<td>cts62</td>
<td>Ref. 22</td>
</tr>
<tr>
<td>MucII4042</td>
<td>cts62 cam repP15A-lac(ZTA)931</td>
<td>Ref. 23</td>
</tr>
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</table>

Transposition of Tn5sac1 from pBRG1410, the plasmid in which it was constructed, to phase λ b221 cl857 Oam29 Pam80, which is integration deficient and defective in replication in a nonsuppressing host, was obtained by growth of the plasmid on Sup+ cells carrying pBRG1410 and then transduction of the Nus− Sup+ strain DB509 to the kanamycin-resistant (KanR) phenotype. The nus mutation in DB509 interferes with the λ N gene function and causes infecting λ phase to form unstable plasmids. The few phase released from such KanR AmpR (ampicillin-sensitive phenotype) transductants contain inserts of the transposon (27). One Tn5sac1-containing λ phase (hereafter called λ::Tn5sac1) was saved for further use.

Transposition of Tn5sac1 to the E. coli chromosome was obtained by infecting the Sup− strains 594 or DB5015 with λ::Tn5sac1 and selecting KanR transductants. Because phase integration and replication are blocked in these strains, most KanR transductants result from Tn5sac1 transposition (9, 12). Transpositions to pBR322 and the related plasmid pBRG1420 were obtained by infecting Sup− strain MC1061 harboring either pBR322 or pBRG1420 with λ::Tn5sac1, extracting plasmid DNAs from pools of KanR transductants, and using these DNAs to transform recipient cells to KanR. MiniMu-mediated generalized transcription was carried out by using MudII4042 and helper phage Mucts62 as described (28).

RESULTS

Rationale for the Design of Tn5sac1. The Tn5sac1 transposon was constructed is diagrammed in Fig. 1. Near the right end of this 4.6-kilobase element is the strong Plac promoter, facing outward so that it can elicit the transcription of adjacent target DNA. Transcription from Plac is blocked by the lacI-encoded repressor but is induced by IPTG, which causes repressor to dissociate from Plac DNA (16). The presence of the lacI gene in Tn5sac1 ensures that Plac will be regulated both in multicopy plasmids (where the repressor made from a single chromosomal gene would be titrated) and in bacterial species that do not contain lacI.

Nineteen base pairs at each end of IS50 are required for its transposition (11, 29, 30). Although transposition of Tn5, which contains inverted repeats of IS50 elements, involves just the outside ends of IS50, Tn5sac1 was built with one outside and one inside end because this combination is generally most active in transposition (ref. 31; K. W. Dodson and D.E.B., unpublished data). Infection of Sup− E. coli strains such as 594 or MC1061 with a λ::Tn5sac1 phase that is defective in replication and integration resulted in KanR transductants at frequencies of about 10−4 per infected cell. As with wild-type Tn5, >90% were free of λ phase (indicating Tn5sac1 transposition) and 2% were auxotrophs.

Conditional Tn5sac1-Induced Tet Mutations in pBR322. To test whether Tn5sac1 could cause conditional mutations, we selected for transposition from λ::Tn5sac1 to plasmid pBR322. Fifty-nine of 600 Tn5sac1 insertions into pBR322 obtained in 16 separate plasmid infections caused a tetracycline-sensitive (TetS) phenotype that was restored to a tetracycline-resistant (TetR) phenotype by growth with IPTG. Restriction mapping indicated that each of 10 independent plasmids contained Tn5sac1 in the tet promoter region, with Plac oriented toward the tet gene (Fig. 2).
Because TnStacl at site 1 is between the end of the tet promoter at pBR322 position 39 and the normal transcriptional start at position 45 (13, 24) (TnStacl sequence begins at position 40), transcription from the tet promoter probably both starts and stops within TnStacl when it is at this site. Because insertions at site 2 are between the start of tet transcription and the start of tet translation (at position 86), the TetR phenotype of the site 2 mutants grown without IPTG shows that TnStacl is polar. More important, the IPTG-dependent TetR phenotype of each of these mutants illustrates that transcription from the P_{lac} promoter of TnStacl can be specifically regulated.

Another test of the ability of TnStacl to conditionally turn on the expression of distal genes involved the use of pBRG1420, a pBR322 derivative that is Tet^{R} because it lacks a functional tet promoter. About one-fifth of TnStacl transposition derivatives of pBRG1420 conferred a Tet^{R} phenotype in the presence of IPTG. Five isolates were characterized by restriction mapping and DNA sequencing—two with strong Tet^{R} phenotypes (large colonies on medium with tetracycline at 12 µg/ml and IPTG) that were also Amp^{S} and three with weak Tet^{R} phenotypes (small colonies on medium with tetracycline at 6 µg/ml and IPTG), one of which was also Amp^{S}. Each contained TnStacl with P_{lac} pointed toward the tet gene. The two insertions that caused a strong IPTG-dependent Tet^{R} phenotype were >400 bp from the normal transcriptional start of tet (sites 10 and 11, Fig. 2 and Table 2); the three that caused a weaker but still IPTG-dependent Tet^{R} phenotype were 900–1300 bp from the tet gene (sites 7–9; Fig. 2 and Table 2). These results show that TnStacl associated with these more distant insertions might reflect the natural polarity of transcription without translation.

**Conditional Lethal TnStacl Insertions in pBR322.** Although the large majority of TnStacl insertions in pBR322 do not affect the growth of host cells, 2% (10 of 500) made cells carrying IPTG-sensitive. Restriction mapping showed that 9 carry TnStacl between the amp gene and the replication origin in the counterclockwise orientation (P_{lac} toward the origin) (sites 5 and 6) and that the 10th carries TnStacl near the end of the tet gene in the opposite (clockwise) orientation (site 4) (Fig. 2). Three of those near the replication origin were sequenced and found to contain TnStacl1 at two sites 11 bp apart (sites 5 and 6, Table 2). An additional conditional lethal mutant (site 3) found in a separate experiment (below) contains TnStacl1 11 bp from site 4 (Table 2; Fig. 2).

The effects of IPTG on cells carrying two plasmids that cause a conditional lethal phenotype were studied in more detail. (i) The colony-forming ability of stationary-phase cells carrying mutants 4 and 5 and of growing cells carrying mutant 4 was reduced on IPTG-containing medium to 3–8% of that on IPTG-free medium. Exponentially growing cells harboring mutant 5 were more sensitive to IPTG (viability of 0.1%) (Table 3). Because the plating medium was antibiotic-free, these results indicate that the plasmids are deleterious during growth with IPTG; the decreased viability is not due to selection against plasmid-free segregants. The addition of tetracycline as well as IPTG to the plating medium, to eliminate cells that had escaped by plasmid loss, led to further reductions in viability (Table 3). (ii) Optical density measurements suggested that mutant 5 interfered more strongly with cell growth than did mutant 4 when IPTG was present, in agreement with plating tests (Fig. 3). (iii) Microscopic observation showed that IPTG caused filamentation of cells carrying mutant 5 but not mutant 4. (iv) Electrophoretic tests showed that growth for 2 hr with IPTG caused a 5- to 10-fold increase in the level of mutant 5 plasmid DNA. IPTG did not stimulate replication of the mutant 4 plasmid nor of a control compatible plasmid, pACYC184, transformed into the pBR322::TnStacl-containing cells for this experiment (Fig.

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**Table 2. Conditional TnStacl insertion mutations in pBR322**

<table>
<thead>
<tr>
<th>Insertion site</th>
<th>Position and orientation*</th>
<th>Phenotype</th>
<th>No. sequenced</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>− IPTG</td>
<td>+ IPTG</td>
</tr>
<tr>
<td>1</td>
<td>31 → 39 (c)</td>
<td>Tet^{S}</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>53 → 61 (c)</td>
<td>Tet^{R}</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1433 → 1441 (c)</td>
<td>wt^{†}</td>
<td>Lethal 1</td>
</tr>
<tr>
<td>4</td>
<td>1444 → 1452 (c)</td>
<td>wt^{†}</td>
<td>Lethal 1</td>
</tr>
<tr>
<td>5</td>
<td>3148 → 3140 (cc)</td>
<td>wt^{†}</td>
<td>Lethal 2</td>
</tr>
<tr>
<td>6</td>
<td>3158 → 3150 (cc)</td>
<td>wt^{†}</td>
<td>Lethal 1</td>
</tr>
<tr>
<td>P_{lac} Plasmid pBRG1420</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3150 → 3158 (c)</td>
<td>Tet^{S}</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>3178 → 3186 (c)</td>
<td>Tet^{R}</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>3495 → 3503 (c)</td>
<td>Tet^{S} Amp^{S}</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>3782 → 3790 (c)</td>
<td>Tet^{S} Amp^{S}</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>3915 → 3923 (c)</td>
<td>Tet^{S} Amp^{S}</td>
<td>1</td>
</tr>
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</table>

*Position corresponds to the 9 bp of pBR322 sequence duplicated by insertion. Sequences were determined by using the following oligonucleotides as primers: A and B, site 1; A and C, site 2; D, sites 3 and 4; C and D, sites 5 and 6; and D, sites 7–11. The orientations (c) and (cc) designate P_{lac} pointing clockwise and counterclockwise, respectively, on the standard pBR322 map (Fig. 2) (13). Insertions 6 and 7 are actually at the same site (they duplicate the same 9 bp) but are in opposite orientations.

†Found in a separate screen for IPTG-sensitive isolates in strain TK278 (see text).

‡(wild type) indicates no effect on phenotype.

§Ten independent isolates that conferred an IPTG-sensitive growth phenotype in strain MC1061 were restriction mapped. One contained TnStacl in the clockwise orientation (site 4), while nine contained TnStacl clustered near the replication origin in the counterclockwise orientation, and three were sequenced.

¶Plasmid overreplicates when cells are grown with IPTG (Fig. 4).

**Weak Tet^{R}** is detected by small colony size on medium containing only half the normal concentration of tetracycline (6 µg/ml).
Table 3. Characteristics of IPTG\textsuperscript{5} TnStacl insertion derivatives of pBR322

<table>
<thead>
<tr>
<th>Insertion site</th>
<th>pBR322 position</th>
<th>+ IPTG</th>
<th>+ IPTG/tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1444 → 1452</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>3148 → 3140</td>
<td>6</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Plating was on complex medium with 0.5 mM IPTG. The plating efficiency is expressed relative to plating on IPTG-free complex medium. The plasmids were in strain MC1061.

4). (v) Finally, one host strain, TK278, was not killed by IPTG when it carried mutant 5 even though IPTG stimulated replication of this plasmid (as in Fig. 4). Mutant 4, in contrast, made TK278 IPTG-sensitive. The screening of 500 additional pBR322::TnStacl insertion plasmids in TK278 resulted in just one IPTG\textsuperscript{5} mutation, which, as expected, was near the end of the \( \text{tet} \) gene (site 3; Fig. 2).

Targeting TnStacl Insertion by Activation of a Silent Chromosomal Gene. Strain DB5015 is a tryptophan auxotroph in which \( \text{trpB} \), whose product converts indole to tryptophan, is poorly transcribed because of polar nonsense mutations in the upstream \( \text{trpE} \) gene (19). TnStacl insertions that caused increased expression of \( \text{trpB} \) were obtained by transposition from \( \text{A}::\text{TnStacl} \). Kan\textsuperscript{R} colonies that were Trp\textsuperscript{+} on medium with indole and IPTG constituted about 10\textsuperscript{-4} of all Kan\textsuperscript{R} transposition products. They were Trp\textsuperscript{-} on indole-containing medium that lacked IPTG, indicating that the \( \text{trpB} \) gene was under \( P_{\text{trp}} \) control.

The linkage of TnStacl to the \( \text{trp} \) operon in two of these independent Trp\textsuperscript{+} mutants was tested by generalized transduction with miniMu phage (28). Thirty Kan\textsuperscript{R} transductants of the Trp\textsuperscript{+} strain XW205 made from each miniMu lysate were scored. On medium containing indole, each of the transductants was Trp\textsuperscript{-} in the absence of IPTG and Trp\textsuperscript{-} in the presence of IPTG, indicating tight linkage of TnStacl to the \( \text{trp} \) operon.

The ability of a resident TnStacl element to activate a silent gene by transposition from one chromosomal site to another was tested: Trp\textsuperscript{+} revertants of derivatives of DB5015 that contained TnStacl at uncharacterized chromosomal locations were found at a frequency of about 10\textsuperscript{-7} on medium containing indole and IPTG. In 39 of 90 of these revertants (10–12 from each of eight different TnStacl insertion strains), the Trp\textsuperscript{+} phenotype was IPTG-dependent. Thus, transposition of a resident TnStacl element to new chromosomal locations can contribute significantly to mutational activation of cryptic genes.

TnStacl-Induced IPTG-Responsive Chromosomal Mutations. We have begun isolating conditional \( E. \coli \) mutants by screening colonies in which TnStacl has transposed to the chromosome. Kan\textsuperscript{R} colonies selected on IPTG-containing complex medium at 30\textdegree C were screened for IPTG-dependence on complex medium and on minimal medium at 30\textdegree C and 42\textdegree C. Approximately 1% were IPTG-dependent on at least one medium. Of four colonies tested, one was an auxotroph, but only when IPTG was absent; a second required IPTG for growth at 30\textdegree C but not at 42\textdegree C; while two required IPTG for growth on both complex and minimal medium.

Approximately 0.2% of Kan\textsuperscript{R} colonies selected on IPTG-free medium were sensitive to IPTG. Of six colonies tested, one lysed spontaneously during growth in IPTG-containing complex medium; a second was auxotrophic when IPTG was present; a third showed partial reversal by kanamycin of IPTG sensitivity; a fourth showed induction by IPTG of a mucoid colony phenotype on both complex and minimal medium; and two others were sensitive to IPTG during growth on both complex and minimal medium (T. Tomsanyi, W.-Y.C., L. Ghaitas, and D.E.B., unpublished data).

Fig. 3. IPTG inhibition of growth of strain MC1061 harboring IPTG-sensitive TnStacl insertion mutants of pBR322. Cultures growing exponentially in LB broth with ampicillin at 37\textdegree C were divided, and IPTG was added to one portion of each culture. Δ, Δ, site 4; ○, ●, site 5; ○, ○, without IPTG; ●, ●, with IPTG. All cells were plated on IPTG-free LB medium. The viable count of cells containing mutant 4 remained unchanged during 4 hr of growth in the presence of IPTG, while the viable count of cells containing mutant 5 decreased to 1/10th in 2 hr and then remained relatively constant for the next 2 hr.

FIG. 4. IPTG-induced overreplication of pBR322::TnStacl plasmids. IPTG was added to exponentially growing cells of MC1061 that harbored two compatible plasmids, pBR322 with TnStacl at site 4 or site 5 and pACYC184 as an internal control. Samples were withdrawn for plasmid extraction at the following times after addition of IPTG: 30 min (lane a); 1 hr (lane b); 2 hr (lane c); 3 hr (lane d); 4 hr (lane e). Equal amounts of each extract were then electrophoresed in 0.7% agarose gels. DNA samples from cells not treated with IPTG were indistinguishable from the mutant 4 extracts (no specific pBR322::TnStacl amplification). The diffuse bands below that marked pBR322::TnStacl in the samples from mutant 5 might represent replication intermediates.
DISCUSSION

We have constructed a Tn5-related transposon called TnStacl for the efficient isolation and characterization of conditional mutations. TnStacl contains the strong P_{ac} promoter near one end, facing outward to elicit the transcription of adjacent target DNA, and a lacI gene so that transcription from P_{lac} can be tightly regulated, independent of host-cell genotype. TnStacl should be useful in diverse bacterial species, greatly facilitating the identification of essential and cryptic genes and of gene products important in the virulence of pathogens, metabolic regulation, and other aspects of bacterial growth.

Tests using a PBR322 plasmid target showed that TnStacl, like wild-type Tn5, inserts into numerous sites (Fig. 2) and generates a variety of IPTG-responsive conditional mutations (Table 2). (i) Insertions at two sites between the promoter and the start of tet translation caused a conditionally Tet<sup>+</sup> phenotype (Tet<sup>+</sup> only in the presence of IPTG). This result showed that TnStacl is polar on distal gene expression and illustrates the use of the mobile P_{ac} promoter to conditionally turn on the expression of distal genes. (ii) Insertions upstream of the promoterless tet gene led to an analogous IPTG-dependent Tet<sup>+</sup> phenotype. The level of resistance to tetracycline was inversely correlated with the distance between the P_{ac} promoter and the tet gene in the range of 400–1200 bp. This and similar results with P_{ac} derivatives of the Tn5-related element Tn721, designed for mutagenizing plasmid DNAs (17), show that P_{ac}-containing transposons should be useful in evolutionary studies of cryptic genes. (iii) TnStacl insertions in two places caused IPTG-sensitive growth phenotypes, apparently by different mechanisms: one class resulted from insertion just downstream of the tet gene near a segment that, although generally innocuous in bacteria, seems to inhibit the growth of some eukaryotic cells (33); a second class resulted from insertion just upstream of the plasmid replication origin [IPTG induced overreplication of plasmids of this mutant class (Fig. 4)]. Their lethality might result directly from excessive replication or from the expression of another deleterious plasmid sequence.

The ability of TnStacl to turn on cryptic chromosomal genes was studied by using a strain containing a silenced trpB gene. About 10<sup>−4</sup> of TnStacl transpositions resulted in a conditionally (IPTG-dependent) Trp<sup>+</sup> phenotype, indicating that these insertions placed the trpB gene under P_{ac} control. In addition, about one-third of the spontaneous revertants formed in strains carrying TnStacl resident elsewhere in the genome resulted from TnStacl transposition to the trp region. Thus, the sporadic movement of TnStacl can reveal cryptic genes.

A general screen of TnStacl chromosomal insertions resulted in a wide variety of conditional phenotypes, some reversed by and some caused by the addition of IPTG. These included mutations to auxotrophy, mucoidy, temperature sensitivity, or inviability under nonpermissive conditions. Most IPTG-correctable mutant phenotypes probably result from polar insertions upstream of the target gene. IPTG-sensitive phenotypes probably result from a variety of mechanisms: at some sites IPTG-induced transcription from P_{ac} may cause mutant phenotypes by overproduction of wild-type gene products; at other sites transcription from P_{lac} may inhibit the use of an essential site, as exemplified by the effects of transcription on the mobility of IS50 (34); at still other sites transcription from P_{ac} may reduce gene expression by interfering with the use of a downstream promoter (35), coupled with inefficient translation of the transcript from P_{lac} (36).

The characteristics of TnStacl should make the conditional mutations it generates much easier to analyze than those due to traditional base substitution mutations. Genetic crosses, reversion tests, and gene cloning will be facilitated by its resistance marker. Sites of insertion can be precisely located, and adjacent genes will often be identifiable by genomic sequencing with oligonucleotide primers specific for TnStacl ends (12). Finally, mRNAs or proteins whose absence or inappropriate synthesis cause mutant phenotypes should be easily detected by pulse labeling. Given the broad host range of Tn5 (9), the approach outlined should be applicable in many bacterial species.

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