Tn5tac1, a derivative of transposon Tn5 that generates conditional mutations

(insertion sequence/*Escherichia coli*/plasmid pBR322/*lac* repressor/isopropyl β -D-thiogalactoside)

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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 Tn5tac1 and its use in an innovative strategy for making mutations with conditional phenotypes. The 4.6-kilobase Tn5tac1 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 β -D-thiogalactoside (IPTG)] is absent. Our results show that two unusual conditional mutant phenotypes can result from Tn5tac1 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 Tn5tac1 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, Tn5tac1 (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 β -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 Tn5tac1 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 μ g/ml; kanamycin, 60 μ g/ml; and tetracycline, 12 μ g/ml (or 6 μ g/ml where noted). IPTG was used at 0.5 mM. Standard procedures were used for bacterial growth, plasmid DNA extraction, 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 as directed.

The oligonucleotides used as sequencing primers are: A, 5'-GTATCACGAGGCCCT (pBR322 positions 4334–4348); B, 5'-GCAATTTAACTGTGAT (pBR322 positions 64–49); C, 5'-GATAAGCTGTCAAAC (pBR322 positions 22–8); and D, 5'-CAGAATTCCCGGGGATCCCC. 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 Tn5tac1 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 Tn5tac1 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 β -D-thiogalactoside; Amp^S and Tet^S, ampicillin- and tetracycline-sensitive phenotypes; Tet^R and Kan^R, tetracycline- and kanamycin-resistant phenotypes; IS, insertion sequence.

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FIG. 1. Tn5tac1. This transposon was constructed in a series of standard cloning, linker ligation, and restriction site fill-in steps in the region between the *EcoRI* and *HindIII* sites of pBR322 (11–13). The end sequences (“O” for outer and “I” for inner) correspond to the first 19 base pairs (bp) from each end of insertion sequence 50 (IS50) (sequence below) and were derived from a plasmid closely related to pBRG1305 (11). The Tn5 *kan* gene segment is from pBRG1404 (12). The Tn5 transposase gene (*tnp*) is from a plasmid closely related to pBRG1306 (11). The *tac* promoter (P_{tac}) was obtained as a *BamHI* fragment of plasmid pKK223-3 (14) from Pharmacia. The *lacI* gene was obtained as an *EcoRI* fragment from plasmid pACYC184-*lacI*^q (15) [*lacI*^q allele used directs the synthesis of high levels of repressor and thus ensures that transcription from P_{tac} will be tightly regulated (16)]. Restriction sites and their positions in base pairs from the left (I) end are as follows: E, *EcoRI*, 25 and 4558; C, *Cla*I, 52; S, *Sma*I, 1384 and 4563; A, *Apa*I, 2110; H, *HindIII*, 3165; N, *Not*I, 3666; X, *Xho*I, 3775; and B, *Bam*HI, 4254 and 4565. The entire element is 4593 bp long. The sequence of positions 1–58 at the left (I) end is: 5'-CTGTCTCTTGATCAGATCTCGGGAGGAATTCTCATGTTTGGACAGCTTATCATCGATCC. The sequence of positions 1–76 of the right (O) end (corresponding to positions 4593–4518 from the left end) is 5'-CTGACTCTTATACACAAGTAGGGGATCCCCGGGAATTCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCCA. The underlining indicates sequences complementary to oligonucleotides C and D, respectively, that were used as primers for chain-termination sequencing sites of Tn5tac1 insertion. The sequences at the Tn5tac1 ends were confirmed after the element was constructed and again after it had transposed to pBR322 (Table 2).

Transposition of Tn5tac1 from pBRG1410, the plasmid in which it was constructed, to phage λ b221 c1857 Oam29 Pam80, which is integration deficient and defective in replication in a nonsuppressing host, was obtained by growth of the phage on Sup⁺ cells carrying pBRG1410 and then transduction of the Nus[−] Sup⁺ strain DB5009 to the kanamycin-resistant (Kan^R) phenotype. The *nus* mutation in DB5009 interferes with the λ N gene function and causes infecting λ phage to form unstable plasmids. The few phage

released from such Kan^R Amp^S (ampicillin-sensitive phenotype) transductants contain inserts of the transposon (27). One Tn5tac1-containing λ phage (hereafter called λ ::Tn5tac1) was saved for further use.

Transposition of Tn5tac1 to the *E. coli* chromosome was obtained by infecting the Sup[−] strains 594 or DB5015 with λ ::Tn5tac1 and selecting Kan^R transductants. Because phage integration and replication are blocked in these strains, most Kan^R transductants result from Tn5tac1 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 ::Tn5tac1, extracting plasmid DNAs from pools of Kan^R transductants, and using these DNAs to transform recipient cells to Kan^R. MiniMu phage-mediated generalized transduction was carried out by using MudII4042 and helper phage Mucts62 as described (28).

RESULTS

Rationale for the Design of Tn5tac1. The Tn5tac1 transposon we constructed is diagrammed in Fig. 1. Near the right end of this 4.6-kilobase element is the strong P_{tac} promoter, facing outward so that it can elicit the transcription of adjacent target DNA. Transcription from P_{tac} is blocked by the *lacI*-encoded repressor but is induced by IPTG, which causes repressor to dissociate from P_{tac} DNA (16). The presence of the *lacI* gene in Tn5tac1 ensures that P_{tac} 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, Tn5tac1 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 ::Tn5tac1 phage that is defective in replication and integration resulted in Kan^R transductants at frequencies of about 10^{−4} per infected cell. As with wild-type Tn5, >90% were free of λ phage (indicating Tn5tac1 transposition) and 2% were auxotrophs.

Conditional Tn5tac1-Induced *tet* Mutations in pBR322. To test whether Tn5tac1 could cause conditional mutations, we selected for transposition from λ ::Tn5tac1 to plasmid pBR322. Fifty-nine of 600 Tn5tac1 insertions into pBR322 obtained in 16 separate phage infections caused a tetracycline-sensitive (Tet^S) phenotype that was restored to a tetracycline-resistant (Tet^R) phenotype by growth with IPTG. Restriction mapping indicated that each of 10 independent plasmids contained Tn5tac1 in the *tet* promoter region, with P_{tac} oriented toward the *tet* gene (Fig. 2).

Table 1. Bacterial strains, plasmids, and phage

Strain	Genotype	Reference/source
Bacteria		
BD1527	<i>met hsdR supE supF rpsL</i>	B. Duncan via T. Kazic*
594	<i>sup^o gal rpsL</i>	Ref. 18
DB5015	W3110 <i>trpEam9851, Eam9829</i> Δ (<i>trpA229-tonB</i>)	Ref. 19
G13	<i>nusA supE</i>	S. Adhya†
JM101	F' <i>lacI^q</i>	J. Messing‡
MC1061	F [−] <i>araD139</i> Δ (<i>ara-leu</i>)7697 <i>lacX74 galU galK hsd hsm rpsL</i>	Ref. 20
TK278	Δ (<i>lac-pro</i>) <i>endA1 gyrA96</i> <i>recA1 thi1 hsdR17 supE44</i> <i>relA1</i>	T. Kazic*
XW205	F [−] <i>araC trpR</i> Δ (<i>proB-lac</i>) ϕ 80dIIacAYZO'-delw205	Ref. 21
Phage		
λ	b221 c1857 Oam29 Pam80	This laboratory
Mucts62	<i>cts62</i>	Ref. 22
MudII4042	<i>cts62 cam repP15A-lac</i> (ZYA)931	Ref. 23
Plasmids		
pBR322	Amp ^R Tet ^R	Refs. 13, 24
pBRG1410	Amp ^R Kan ^R	pBR322-derived plasmid containing Tn5tac1; see Fig. 1
pBRG1420	Amp ^R Tet ^S	pBR322 with <i>Bam</i> HI linker insert in filled <i>Hind</i> III site (<i>tet</i> promoter)
pACYC184	Tet ^R Cam ^R	Ref. 13
pACYC184- <i>lacI^q</i>	Tet ^R	Ref. 15

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FIG. 2. Map of Tn5tac1 insertions in pBR322 and in its derivative pBRG1420, which is Tet^S because of inactivation of the -10 region of P_{tac} . Numbers refer to sites listed in Table 2. Arrows indicate the orientation of Tn5tac1 (direction of transcription from P_{tac}). Arrows outside the circle depict insertions in pBR322, and arrows inside the circle depict insertions in pBRG1420.

The sites of Tn5tac1 insertion in these 10 conditional Tet^R mutants were determined by double-stranded DNA sequencing (see legend to Table 2). Of the 10, 8 contained Tn5tac1 at just one site (site 1), and the other 2 contained Tn5tac1 at a second site (site 2) (Table 2, Fig. 2). Site 1 had previously been identified as a major hotspot for the insertion of wild-type Tn5 (32). The sequencing of both Tn5tac1-pBR322 junctions in each mutant showed that Tn5tac1, like wild-type Tn5, generates 9-bp target sequence duplications.

Table 2. Conditional Tn5tac1 insertion mutations in pBR322

Insertion site	Position and orientation*	Phenotype		No. sequenced
		– IPTG	+ IPTG	
Wild-type pBR322				
1	31 → 39 (c)	Tet ^S	Tet ^R	8
2	53 → 61 (c)	Tet ^S	Tet ^R	2
3 [†]	1433 → 1441 (c)	wt [‡]	Lethal	1
4 [§]	1444 → 1452 (c)	wt [‡]	Lethal	1
5 [§]	3148 → 3140 (cc)	wt [‡]	Lethal	2
6 ^{¶§}	3158 → 3150 (cc)	wt [‡]	Lethal	1
<i>P</i> _{tet} plasmid pBRG1420				
7	3150 → 3158 (c)	Tet ^S	Weak Tet ^{R**}	1
8	3178 → 3186 (c)	Tet ^S	Weak Tet ^R	1
9	3495 → 3503 (c)	Tet ^S Amp ^S	Weak Tet ^R Amp ^S	1
10	3782 → 3790 (c)	Tet ^S Amp ^S	Tet ^R Amp ^S	1
11	3915 → 3923 (c)	Tet ^S Amp ^S	Tet ^R Amp ^S	1

*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 D, 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_{tac} 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).

‡wt (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 Tn5tac1 in the clockwise orientation (site 4), while nine contained Tn5tac1 clustered near the replication origin in the counterclockwise orientation, and three were sequenced.

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

||This plasmid is Tet^S because of an insertion in the wild-type *tet* promoter.

**Weak Tet^R is detected by small colony size on medium containing only half the normal concentration of tetracycline (6 μ g/ml).

Because Tn5tac1 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) (Tn5tac1 sequence begins at position 40), transcription from the *tet* promoter probably both starts and stops within Tn5tac1 when it is at this site. Because inserts at site 2 are between the start of *tet* transcription and the start of *tet* translation (at position 86), the Tet^S phenotype of the site 2 mutants grown without IPTG shows that Tn5tac1 is polar. More important, the IPTG-dependent Tet^R phenotype of each of these mutants illustrates that transcription from the P_{tac} promoter of Tn5tac1 can be specifically regulated.

Another test of the ability of Tn5tac1 to conditionally turn on the expression of distal genes involved the use of pBRG1420, a pBR322 derivative that is Tet^S because it lacks a functional *tet* promoter. About one-fifth of Tn5tac1 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 μ g/ml and IPTG) that were also Amp^S and three with weak Tet^R phenotypes (small colonies on medium with tetracycline at 6 μ g/ml and IPTG), one of which was also Amp^S. Each contained Tn5tac1 with P_{tac} 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). The low-level Tet^R associated with these more distant insertions might reflect the natural polarity of transcription without translation.

Conditional Lethal Tn5tac1 Insertions in pBR322. Although the large majority of Tn5tac1 insertions in pBR322 do not affect the growth of host cells, 2% (10 of 500) made cells carrying them IPTG-sensitive. Restriction mapping showed that 9 carry Tn5tac1 between the *amp* gene and the replication origin in the counterclockwise orientation (P_{tac} toward the origin) (sites 5 and 6) and that the 10th carries Tn5tac1 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 Tn5tac1 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 Tn5tac1 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::Tn5tac1-containing cells for this experiment (Fig.

Table 3. Characteristics of IPTG^S Tn5tac1 insertion derivatives of pBR322

Insertion site	pBR322 position	Efficiency of plating, * %			
		+ IPTG		+ IPTG/tetracycline	
		Stationary	Growing	Stationary	Growing
4	1444 → 1452	8	3	0.1	0.3
5	3148 → 3140	6	0.1	0.01	0.0006

*Platings were 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::Tn5tac1 insertion plasmids in TK278 resulted in just one IPTG^S mutation, which, as expected, was near the end of the *tet* gene (site 3; Fig. 2).

Targeting Tn5tac1 Insertion by Activation of a Silent Chromosomal Gene. Strain DB5015 is a tryptophan auxotroph in which *trpB*, whose product converts indole to tryptophan, is poorly transcribed because of polar nonsense mutations in the upstream *trpE* gene (19). Tn5tac1 insertions that caused increased expression of *trpB* were obtained by transposition from λ ::Tn5tac1. Kan^R colonies that were Trp⁺ on medium with indole and IPTG constituted about 10⁻⁴ of all Kan^R transposition products. They were Trp⁻ on indole-containing medium that lacked IPTG, indicating that the *trpB* gene was under *P*_{tac} control.

The linkage of Tn5tac1 to the *trp* operon in two of these independent Trp⁺ mutants was tested by generalized transduction with miniMu phage (28). Thirty Kan^R transductants of the Trp⁺ strain XW205 made from each miniMu lysate were scored. On medium containing indole, each of the transductants was Trp⁻ in the absence of IPTG and Trp⁺ in the presence of IPTG, indicating tight linkage of Tn5tac1 to the *trp* operon.

The ability of a resident Tn5tac1 element to activate a silent gene by transposition from one chromosomal site to another was tested: Trp⁺ revertants of derivatives of DB5015 that

contained Tn5tac1 at uncharacterized chromosomal locations were found at a frequency of about 10⁻⁷ on medium containing indole and IPTG. In 39 of 90 of these revertants (10–12 from each of eight different Tn5tac1 insertion strains), the Trp⁺ phenotype was IPTG-dependent. Thus, transposition of a resident Tn5tac1 element to new chromosomal locations can contribute significantly to mutational activation of cryptic genes.

Tn5tac1-Induced IPTG-Responsive Chromosomal Mutations. We have begun isolating conditional *E. coli* mutants by screening colonies in which Tn5tac1 has transposed to the chromosome. Kan^R colonies selected on IPTG-containing complex medium at 30°C were screened for IPTG-dependence on complex medium and on minimal medium at 30°C and 42°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°C but not at 42°C; while two required IPTG for growth on both complex medium and minimal medium.

Approximately 0.2% of Kan^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. Tomcsanyi, W.-Y.C., I. Ghattas, and D.E.B., unpublished data).

FIG. 3. IPTG inhibition of growth of strain MC1061 harboring IPTG-sensitive Tn5tac1 insertion mutants of pBR322. Cultures growing exponentially in LN broth with ampicillin at 37°C were divided, and IPTG was added to one portion of each culture. Δ , $\bullet$, site 4; $\circ$, $\bullet$, site 5; $\circ$, Δ , without IPTG; $\bullet$, Δ , with IPTG. All cells were plated on IPTG-free LN 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::Tn5tac1 plasmids. IPTG was added to exponentially growing cells of MC1061 that harbored two compatible plasmids, pBR322 with Tn5tac1 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::Tn5tac1 amplification). The diffuse band below that marked pBR322::Tn5tac1 in the samples from mutant 5 might represent replication intermediates.

DISCUSSION

We have constructed a Tn5-related transposon called Tn5tac1 for the efficient isolation and characterization of conditional mutations. Tn5tac1 contains the strong P_{tac} promoter near one end, facing outward to elicit the transcription of adjacent target DNA, and a *lacI* gene so that transcription from P_{tac} can be tightly regulated, independent of host-cell genotype. Tn5tac1 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 Tn5tac1, 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^R phenotype (Tet^R only in the presence of IPTG). This result showed that Tn5tac1 is polar on distal gene expression and illustrates the use of the mobile P_{tac} promoter to conditionally turn on the expression of distal genes. (ii) Insertions upstream of a promoterless *tet* gene led to an analogous IPTG-dependent Tet^R phenotype. The level of resistance to tetracycline was inversely correlated with the distance between the P_{tac} promoter and the *tet* gene in the range of 400–1200 bp. This and similar results with P_{tac} derivatives of the Tn3-related element Tn721, designed for mutagenizing plasmid DNAs (17), show that P_{tac} -containing transposons should be useful in evolutionary studies of cryptic genes. (iii) Tn5tac1 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 Tn5tac1 to turn on cryptic chromosomal genes was studied by using a strain containing a silenced *trpB* gene. About 10⁻⁴ of Tn5tac1 transpositions resulted in a conditionally (IPTG-dependent) Trp⁺ phenotype, indicating that these insertions placed the *trpB* gene under P_{tac} control. In addition, about one-third of the spontaneous revertants formed in strains carrying Tn5tac1 resident elsewhere in the genome resulted from Tn5tac1 transposition to the *trp* region. Thus, the sporadic movement of Tn5tac1 can reveal cryptic genes.

A general screen of Tn5tac1 chromosomal insertions resulted in a 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_{tac} may cause mutant phenotypes by overproduction of wild-type gene products; at other sites transcription from P_{tac} 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_{tac} may reduce gene expression by interfering with the use of a downstream promoter (35), coupled with inefficient translation of the transcript from P_{tac} (36).

The characteristics of Tn5tac1 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 Tn5tac1 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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