# TRANSDUCTIONAL INSTABILITY OF Tn*5*-INDUCED MUTATIONS: GENERALIZED AND SPECIALIZED TRANSDUCTION OF Tn*5* BY BACTERIOPHAGE P1

CLAIRE M. BERG,*[,1] CARMEN A. GRULLÓN,* AOQUAN WANG,*[,2] WILLIAM A. WHALEN* AND DOUGLAS E. BERG[†]

* *Biological Sciences Group (Box U-131), The University of Connecticut, Storrs, Connecticut 06268, and* [†] *Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110*

Manuscript received March 2, 1983
Revised copy accepted June 23, 1983

## ABSTRACT

Generalized transduction is commonly used to move transposon-induced mutations among bacterial strains by selecting for inheritance of a transposon-encoded resistance determinant. Although complete cotransduction of the resistance determinant and the chromosomal mutation might be expected, it is often found that when Tn*5*(Kan) insertion mutations are transduced by bacteriophage P1 most of the nonmutant kanamycin-resistant transductants are due to specialized transduction of Tn*5*. Such P1::Tn*5* specialized transducing phage are not found when a mutant Tn*5* element lacking a functional transposase is employed.

BECAUSE transposable elements are ubiquitous DNA segments which move to new locations in a genome in the absence of homology, they are important to bacterial evolution and extraordinarily valuable as genetic tools. The drug resistance transposon Tn*5* contains terminal inverted repeats of the insertion sequence IS*50* bracketing a central segment containing a kanamycin-neomycin (Kan)-resistant gene (BERG *et al.* 1980). Tn*5* moves at a relatively high frequency and inserts into many genomic sites in *Escherichia coli* and other Gram-negative bacteria (BERG and BERG 1981, 1983). When Tn*5*-induced mutations are introduced into a new strain, linkage between the selected Kan$^r$ trait and the bacterial mutation is usually incomplete (BERG and BERG 1981). We show that this is due to the transposase-catalyzed movement of Tn*5* to other sites, usually into the P1 genome.

## MATERIALS AND METHODS

*Phage and bacteria:* P1Cm*c*1·100 (ROSNER 1972), herein called P1, was used in all transductions. This phage carries Tn*9*, which encodes chloramphenicol (Cam) resistance and a temperature-sensitive allele of the prophage repressor gene, *c*1. Tn*5*-410 is a derivative of Tn*5*, which encodes *trpE*, but not *kan*, (Figure 1) and can replace Tn*5* by homologous recombination between the ends of the elements (BERG *et al.* 1980). Five *lac*::Tn*5* mutations in the DB1506 (F′*lac*::Tn*5*) series and

[1] To whom correspondence and reprint requests should be addressed.
[2] Permanent address: Institute of Microbiology, Academia Sinica, Beijing, China.

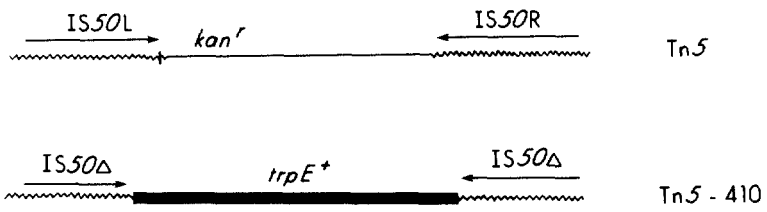


FIGURE 1.—The structures of Tn*5* and Tn*5*-410 (BERG *et al.* 1980; MEYER, BOCH and SHAPIRO 1979). The Δ symbol indicates deletion of the interior 318 bp of IS*50*L and IS*50*R.

the corresponding *lac*::Tn*5*-410(Trp) replacements (BERG *et al.* 1980) were employed. Each *lac*::Tn*5* and *lac*Tn*5*-410 mutation was transduced to the chromosome of DB1470 (Δ*trpE5 trpR tna*) to avoid complicating the analysis with transductionally shortened F′ factors which were found when the donor marker was on F′*lac* (C. A. GRULLÓN and C. M. BERG, unpublished results). CBK700 (*ilvE720*::Tn*5* Δ*proB-lac thyA*) (WHALEN and BERG 1982) was used as the donor in some experiments, and DB1470 or DB1972-2 (*recA*Δ*306*::Tn*10* Δ*rac* Δ*trpE5 trpR tna*) was used as the recipient. Lysogens were isolated by infection with P1 at 30°.

*Media:* Lennox broth (LB) and agar (LA) plus 10 μg/ml of thymine and Vogel and Bonner medium E plus 10 μg/ml of thymine were used as the complex and minimal media, respectively (CARO and BERG 1971). When transductants were selected on complex medium, sodium chloride was replaced by 5 μg/ml of sodium citrate (LA+Cit). Difco MacConkey medium plus 10 μg/ml of thymine was used as the Lac indicator medium. The complex media were supplemented with Cam (25 μg/ml) or Kan (30 μg/ml) as required. Medium E was supplemented as required (CARO and BERG 1971) or with Difco casamino acids (CA) (0.5%).

*Transduction and transductant testing:* Each lysate was obtained from a separate single colony of a P1 lysogen using the method of M. HOWE (personal communication). Phage production was induced by 30-min growth with aeration in LB at 42° followed by growth at 37° until clearing was observed (usually 60–90 min). The bacterial debris was removed by centrifugation, after treating with chloroform. Titers of $\sim 10^{10}$ plaque-forming units/ml were routinely obtained.

Recipients were infected at a multiplicity of infection of <1.0. After 30–45 min at 30°, appropriate dilutions were plated on LA + Cit plus Kan or on medium E plus CA. Except where noted, plates were incubated at 30°.

Kan$^r$ specialized transducing phage were characterized by spotting 0.05 and 0.0005 ml of freshly prepared lysates on a LA plate spread with $\sim 5 \times 10^7$ cells of a stationary phase Kan$^s$ culture. These master plates were incubated overnight at 30°, replica plated to LA containing Cam or Kan and incubated for 24 h at 30°. A patch of growth on the antibiotic plate reflected the presence of the corresponding transposon in P1.

## RESULTS

*Analysis of transductants:* From 25 to 85% of Kan$^r$ transductants of Tn*5*-induced mutations, depending on the donor allele, failed to inherit the original Tn*5* insertion mutation. For several mutants the tightness of linkage was found to be reduced by infection at a phage multiplicity of >0.1 and to be increased by infection at 42° (data not shown).

The properties of Kan$^r$ transductants that did not inherit the chromosomal allele were examined in more detail. Phage grown on CBK700 (*ilvE*::Tn*5*) was used, and of 271 Kan$^r$ Ilv$^+$ transductants, 267 were Cam$^r$. One hundred and nineteen of 134 tested released viable phage upon thermal induction. One hundred and fifteen of these were high frequency transducers of Kan$^r$ as well as Cam$^r$. Eighty-six percent (115 of 134) of the Kan$^r$ prototrophs appear, therefore, to carry P1 plasmids with Tn*5* inserted into a region of the P1

genome not needed for plasmid maintenance or for lytic growth. Thus, Kan$^r$ transductants from a Tn*5* insertion strain fall into two main classes: generalized transductants in which Tn*5* remains linked to its original chromosomal site and specialized transductants harboring P1::Tn*5* plasmids. The formation of specialized transducing phage is, therefore, mostly responsible for decreased linkage between the original mutation and Tn*5*: conditions that favor lysogenization (infection with more than one phage per cell at the permissive temperature) reduce the frequency of linked inheritance of Tn*5* and the chromosomal mutation.

*Transposition of Tn5 to P1 requires a functional transposase:* Experiments similar to those just presented were carried out using P1 grown on *lac*::Tn*5* insertion strains. A variable fraction (46% overall) of the Kan$^r$ transductants were Cam$^s$, temperature-resistant true transductants which received the donor Lac$^-$ allele (Table 1A). In contrast, most of the Kan$^r$ transductants that were Lac$^+$ were also Cam$^r$. All 25 Lac$^+$, Cam$^r$ transductants tested (five from each transduction) were found to be temperature sensitive and able to release Kan$^r$ specialized transducing phage. Of the 16 Kan$^r$ transductants that were Lac$^+$ and Cam$^s$, 15 (1.3% of the total in Table 1A) were temperature resistant, indicating that they probably arose by transposition of Tn*5* to new chromosomal sites (transposition could have occurred in the donor prior to pickup of a generalized transducing fragment or in the recipient).

Transposition-defective *lac*::Tn*5*-410(Trp) strains closely related to the *lac*::Tn*5* strains were used to test the role of transposase in specialized transduction. Trp$^+$ transductants were of only one type: Lac$^-$, Cam$^s$ (Table 1B). None of these transductants were temperature sensitive. Thus, Tn*5*-410 is unable to transpose to P1.

*Generalized transduction of Tn5 requires a* recA *function:* When P1, grown on F$^-$ *lac*::Tn*5* insertion strains, was used to transduce a *recA* strain to Kan$^r$, we obtained only about 20% of the number of transductants found when a *recA*$^+$ recipient was used, but all were Lac$^+$. Three hundred and eighty-seven of 443 (87%) were Cam$^r$ and temperature sensitive for growth, reflecting transposition of Tn*5* to the P1 genome and the establishment of P1::Tn*5* plasmids. The absence of Lac$^-$ transductants reflected the absence of generalized transduction in *recA* recipients (CLARK 1973).

When P1 grown on *lac*::Tn*5*-410 insertion strains was used to transduce a *recA* strain to Trp$^+$, no transductants were found under conditions that would have yielded at least 100 Trp$^+$ transductants for each of the five strains if a *rec*$^+$ recipient had been used. Thus, when a transposition-defective transposon and a *recA*$^-$ recipient are used, neither generalized nor specialized transductants can be obtained.

## DISCUSSION

Experiments presented here demonstrate that linkage between Tn*5* and a Tn*5*-induced chromosomal mutation is reduced upon generalized transduction by P1 and selection for the transposon-encoded kanamycin-resistant determi-

TABLE 1

*Characteristics of Kan$^R$ or Trp$^+$ transductants using P1 grown on* lac::*Tn5(Kan) and* lac::*Tn5-410(Trp) strains*[a]

| Insertion site | Lac$^-$ | | Lac$^+$ | | Total |
|---|---|---|---|---|---|
| | Cam$^s$[b] | Cam$^r$ | Cam$^s$[b] | Cam$^r$[c] | |
| A. *lac*::Tn*5*(Kan) host | | | | | |
| *lacY141* | 102 | 0 | 3 | 171 | 276 |
| *lacY148* | 129 | 0 | 7 | 186 | 322 |
| *lacZ155* | 195 | 0 | 2 | 106 | 303 |
| *lacZ202* | 50 | 0 | 0 | 50 | 100 |
| *lacZ217* | 64 | 0 | 4 | 107 | 175 |
| B. *lac*::Tn*5*-410(Trp) host | | | | | |
| *lacY141* | 194 | 0 | 0 | 0 | 194 |
| *lacY148* | 143 | 0 | 0 | 0 | 143 |
| *lacZ155* | 169 | 0 | 0 | 0 | 169 |
| *lacZ202* | 169 | 0 | 0 | 0 | 169 |
| *lacZ217* | 162 | 0 | 0 | 0 | 162 |

[a] The recipient used was DB1470 (*lac*$^+$ Δ*trp*).
[b] Temperature resistant (do not contain P1).
[c] Temperature sensitive (contain P1::Tn*5*).

nant because Tn*5* moves at a frequency comparable to the frequency of generalized transduction, usually to the P1 genome. This is dependent upon the Tn*5*-encoded transposase (Table 1). Our data suggest that the frequency of Tn*5* transposing to the P1 genome and then establishment of P1 as a plasmid is close to the frequency of a specific fragment of host DNA being incorporated into a generalized transducing phage and then being inserted into the recipient chromosome to give a true transductant (Table 1A; C. M. BERG and C. A. GRULLÓN, unpublished results).

The same range in prototrophic frequencies is found whether Tn*5*-induced (this work) or Tn*10*-induced (KLECKNER *et al.* 1978; A. WANG and C. M. BERG, unpublished results) *E. coli* mutations are transduced by P1 or whether Tn*5*-induced *Salmonella typhimurium* mutations are transduced by P22 (SHAW, BERG and SOBOL 1980; W. A. WHALEN and C. M. BERG, unpublished results). Since Tn*10* generally transposes less efficiently than does Tn*5*, the high recovery of P1::Tn*10* elements may reflect a hot spot for Tn*10* insertion in P1.

When a transposon-induced mutation is being moved from strain to strain, the loss of complete linkage between the transposon's resistance determinant and the mutation is only a nuisance if readily detectable mutations are being moved but can be a serious hindrance if the mutation is difficult to assay or silent in certain backgrounds. In such cases, a number of strategies can be employed to eliminate or reduce the frequency of nonchromosomal transductants. Among such strategies are use of a transposition-defective element (Table 1B), infection with a low phage multiplicity (RESULTS), selection of transductants that do not inherit phage markers (Table 1A), infection under conditions in which the phage cannot survive (RESULTS); infection of a strain

already lysogenic for P1, use of a virulent transducing phage and/or selection for a linked marker and screening of Kan$^r$ cotransductants.

Presented at the 51st meeting of the Genetics Society of America [Genetics **100**(suppl): s17–18, 1982]. This work was supported by Public Health Services grant AI-14278, American Cancer Society grant MV-85 and National Science Foundation grant PCM 8104660 to C. M. B., and by Public Health Service grant AI-18980 to D. E. B. W. A. W. was supported by Public Health Service Genetics Training grant 5T32 GM-07584.

## LITERATURE CITED

BERG, C. M. and D. E. BERG, 1981 Bacterial transposons. pp. 107–116. In: *Microbiology-1981*, Edited by D. SCHLESSINGER. American Society for Microbiology, Washington, D.C.

BERG, D. E. and C. M. BERG, 1983 The prokaryotic transposable element Tn5. Biotechnology **1:** 417–435.

BERG, D. E., C. EGNER, B. J. HIRSCHEL, J. HOWARD, L. JOHNSRUD, R. A. JORGENSEN and T. D. TLSTY, 1980 Insertion, excision, and inversion of Tn5. Cold Spring Harbor Symp. Quant. Biol. **45:** 115–123.

CARO, L. and C. M. BERG, 1971 P1 transduction. Methods Enzymol. **12(C):** 444–458.

CLARK, A. J., 1973 Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7:** 67–86.

KLECKNER, N., D. F. BARKER, D. G. ROSS and D. BOTSTEIN, 1978 Properties of the translocatable tetracycline-resistant element Tn*10* in *Escherichia coli* and bacteriophage lambda. Genetics **90:** 427–450.

MEYER, R., G. BOCH and J. SHAPIRO, 1979 Transposition of DNA inserted into deletions of the Tn*5* kanamycin resistance element. Mol. Gen. Genet. **171:** 7–13.

ROSNER, J. L., 1972 Formation, induction, and curing of bacteriophage P1 lysogens. Virology **49:** 679–689.

SHAW, K. J., C. M. BERG and T. J. SOBOL, 1980 *Salmonella typhimurium* mutants defective in acetohydroxy acid synthases I and II. J. Bacteriol. **141:** 1258–1263.

WHALEN, W. A. and C. M. BERG, 1982 Analysis of an *autA::Mu d1*(Ap *lac*) mutant: metabolic role of transaminase C. J. Bacteriol. **150:** 739–746.

Corresponding editor: G. MOSIG