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Absence of DNA Sequences Homologous to Transposable Element Tn5 (Kan) in the Chromosome of *Escherichia coli* K-12

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The DNA hybridization procedure of Southern has been used to search for homology between the transposable kanamycin resistance determinant Tn5 and sequences in the chromosome of *Escherichia coli* K-12. No homology was detected under conditions in which a segment homologous to 5% or more of the 5,300-base pair Tn5 element would have been seen.

Transposable elements (transposons) are specialized DNA segments able to insert into many different sites in a genome (for a review, see references 3 and 15). Transposition, unlike classical crossing-over, does not depend strongly on functions involved in generalized recombination or on extensive DNA sequence homology.

Several classes of transposons have been recognized. The smallest transposons, designated IS sequences, are usually less than 2,000 base pairs (2 kb) long and contain only genes which encode functions related to transposition. The insertion of an IS sequence into a gene is a frequent cause of spontaneous mutation.

The larger "Tn" elements appear to be gene clusters in which genes with functions unrelated to transposition are flanked by IS-like sequences. Resistance genes in naturally occurring bacterial plasmids are often found in such Tn elements. Several findings have led to the current hypothesis that many Tn elements contain pairs of IS elements which provide the genes and sites necessary for transposition. First, bacteriophage Mu, which itself behaves like an IS element, can promote the transposition of bacterial genes to new sites when Mu functions are expressed; the transposed genes are always flanked by a pair of Mu prophages (5). Second, the sequences repeated directly at the ends of the chloramphenicol resistance transposon Tn9 are homologous to IS1 (11); these same IS1 sequences flank a gene encoding enterotoxin in a newly discovered transposon (M. So, personal communication). This last pair of findings also indicates that the same IS sequences may be used when different Tn elements are formed.

This note concerns Tn5, a transposable kanamycin resistance determinant derived from JR67, a resistance plasmid first found in *Klebsiella* and transferred to *Escherichia coli* (1, 2). Tn5 is 5.3 kb long and contains 1.45-kb terminal inverted repetitions. Tn5 transposes to new sites at high frequencies, mutates genes into which it inserts, and can insert into many different sites within a single gene. Mutations induced by Tn5 are polar, stable, nonleaky, and insensitive to allele-specific suppressors (1).

The inverted repetitions of Tn5 are similar in size to the *E. coli* IS sequences IS2, IS3, and IS4 (15). Since direct tests of possible homology between the inverted repetitions of Tn5 and any of these IS sequences have not been reported, we have used a modification (10) of the Southern (14) hybridization procedure to examine this question. Our results, given below, indicate that there is no significant homology between Tn5 and the *E. coli* chromosome.

Purified DNAs of wild-type *E. coli* and a derivative containing Tn5 near the *lac* operator were digested with restriction endonucleases and subjected to electrophoresis in agarose gels (Fig. 1). The DNAs were denatured in alkali, neutralized, and transferred to sheets of nitrocellulose. Strips cut from these nitrocellulose sheets were hybridized to ^{32}P-labeled probe DNAs and autoradiographed. We draw the following conclusions from the autoradiographs in Fig. 2.

(i) There is no detectable homology between Tn5 and the DNA of the *E. coli* chromosome (homology extending over 200 bases would have been readily detected). Therefore, the inverted repetitions of Tn5 cannot be IS2, IS3, or IS4.

(ii) In the endonuclease *Eco*RI digest of the

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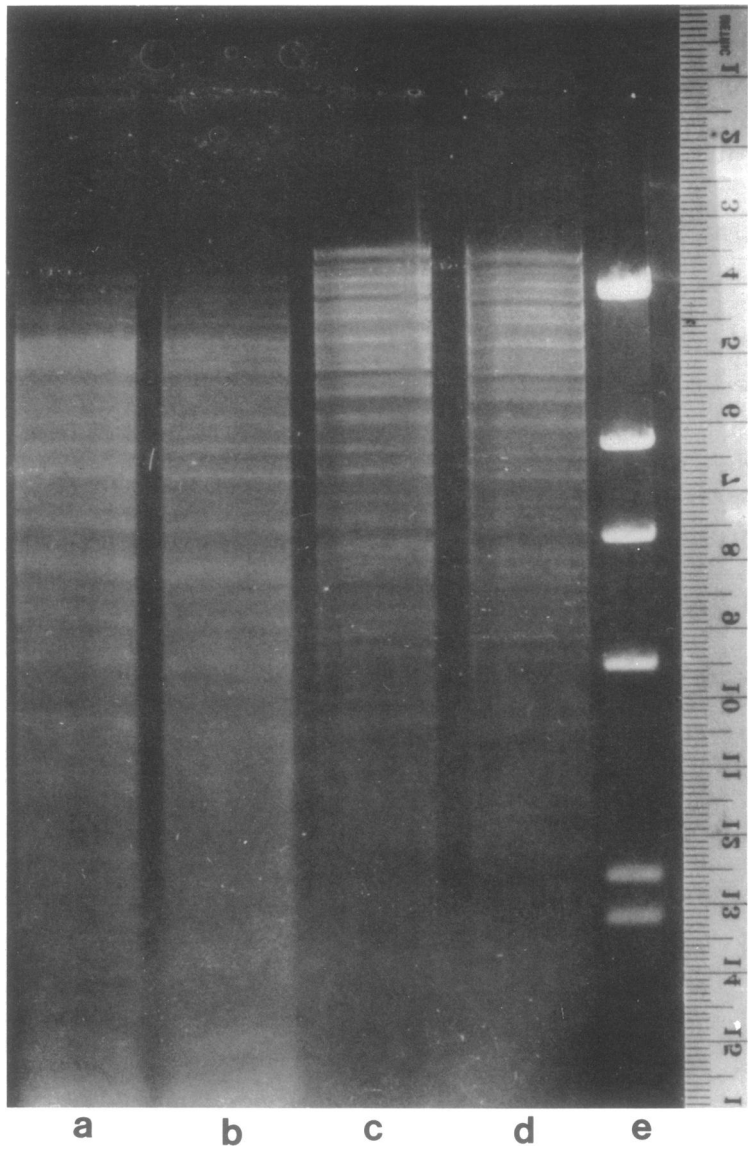

FIG. 1. *Agarose gel electrophoresis of EcoRI/BstEI double digests and EcoRI single digests of E. coli DNAs. The DNAs of E. coli K-12 strain DB1161 and strain DB1162-36 (isogenic with DB1161 except for a Tn5 insertion mutation mapped genetically near the lac operon promoter [1]) were purified by using lysozyme, ethylenediaminetetraacetic acid, and sodium dodecyl sulfate lysis and phenol extraction. The DNAs were digested with endonucleases EcoRI (purchased from Miles Laboratories, Inc.) or EcoRI followed by BstEI (a gift of Richard Meager) in 100 mM tris(hydroxymethyl)aminomethane (pH 7.6)–6 mM $MgCl_2$–100 mM NaCl at 37°C. The digested DNAs were loaded at a concentration of 10-μg/cm slot width in 0.7% agarose gels buffered with 0.08 M tris(hydroxymethyl)aminomethane–0.04 M sodium acetate–0.004 M ethylenediaminetetraacetic acid (pH 8.0), subjected to electrophoresis at 1.5 V/cm, stained with ethidium bromide, and photographed. The DNA samples from left to right are: (a) EcoRI- and BstEI-digested 1162-36, (b) EcoRI- and BstEI-digested 1161, (c) EcoRI-digested 1162-36, (d) EcoRI-digested 1161, (e) HindIII-digested λ^+ (fragment sizes, 23, 9.8, 6.6, 4.5, 2.5, and 2.2 kb) (12). Tn5 is not cleaved by EcoRI (2), but is cleaved by BstEI (R. Jorgensen, personal communication). There is an EcoRI cleavage site near the 3′ end of lacZ (4).*

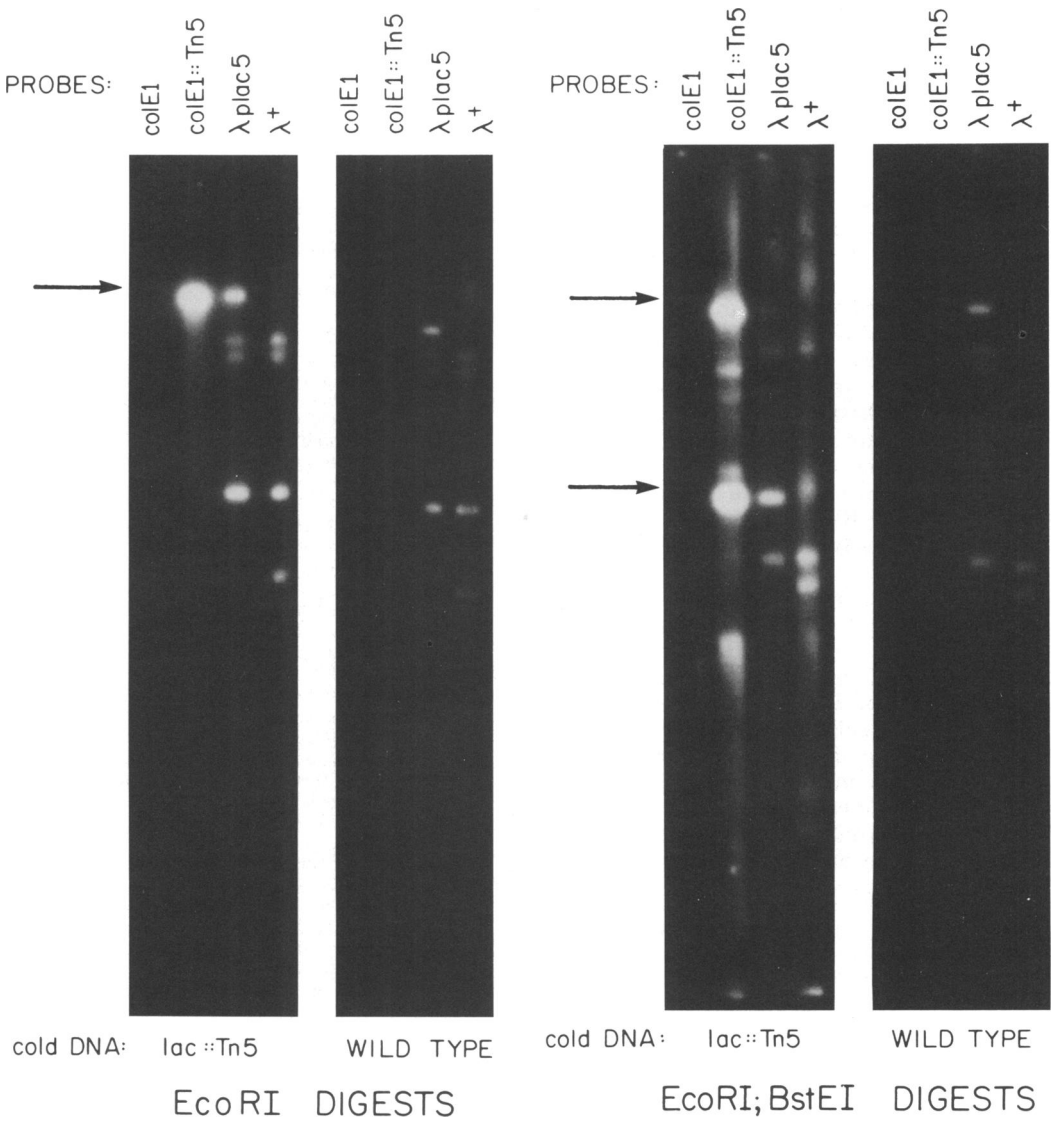

FIG. 2. *Autoradiographs of Southern transfers hybridized with various ^{32}P-labeled probe DNAs. The DNA bands derived by endonuclease treatment of E. coli wild type and lac::Tn5 DNAs and separated by electrophoresis (Fig. 1) were transferred to cellulose nitrate sheets and hybridized to radioactive probe DNAs (as indicated) by a modification (10) of the Southern (14) method. Probe DNAs (plasmids ColE1 wild type [8] and ColE1::Tn5 generated by transposition of Tn5 in vivo [R. Jorgensen, manuscript in preparation] and phages λcI857Sam7 and λplac5cI857Sam7 [9]) were labeled in vitro by nick translation (13) to specific activities of 5 × 10^6 to 11 × 10^6 cpm/μg. Hybridization was carried out with 10^6 cpm of heat-denatured probe DNA per ml. Cellulose nitrate strips were wetted with reaction buffer containing 20 μg of denatured salmon sperm DNA per ml before adding labeled DNA. Hybridization reactions were incubated in 0.3 M sodium chloride–0.03 trisodium citrate (2× SSC) with 20 μg of denatured salmon sperm DNA per ml as carrier at 67°C for 18 h. The strips were then washed extensively in 2× SSC at 67°C, dried, and subjected to autoradiography for 4 weeks. The arrows indicate the sites of hybridization between lac::Tn5 and labeled ColE1::Tn5 probe DNAs.*

lac::Tn*5* insertion mutant, the band of about 28 kb which hybridizes to the ^{32}P-labeled *lac* probe also hybridizes to the ^{32}P-labeled Tn*5* probe. In the *Eco*RI/*Bst*EI double digest, two major bands of about 21 and 6.5 kb hybridize with Tn*5* DNA; the 6.5-kb band binds much more *lac* DNA than does the larger fragment. These observations are consistent with the location of

Tn*5* deduced by deletion mapping (1) and with the known distribution of *Eco*RI and *Bst*EI cleavage sites in Tn*5* and in *lac* (see legend to Fig. 1). The pattern is complicated somewhat by minor Tn*5*-specific bands between the major 6.5-kb and 21-kb Tn*5* bands. Although *Bst*EI usually cleaves at the 6-base pair sequence GGATCC/CCTAGG, occasional *Bst*EI cleavage at the 4-base pair sequence GATC/CTAG results in minor bands (R. Meagher, personal communication).

(iii) The digests of both bacterial DNAs contain fragments which hybridize to the λ probe even though these bacterial strains are not λ immune, do not produce phage after inducing doses of UV irradiation, and do not complement λ phage which are mutant in replication, cell lysis, or morphogenesis. These λ-related sequences reflect the presence of cryptic lambdoid phages in *E. coli* K-12 (which have been demonstrated previously in genetic marker rescue experiments [6, 7]).

(iv) There is no detectable homology between plasmid ColE1 and *E. coli* chromosomal DNA.

In conclusion, we find no sequences in the chromosome of *E. coli* which are homologous to as much as 5% of Tn*5*. We expect that IS sequences with homology to the inverted repeats of Tn*5* will be found in other bacterial species—perhaps in the *Klebsiella* group in which the R factor carrying Tn*5* was discovered or in the as yet unknown bacterial species in which the kanamycin resistance determinant of Tn*5* first evolved.

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