ESCHERICHIA COLI K-12 AUXOTROPHS INDUCED BY INSERTION OF THE TRANSPOSABLE ELEMENT Tn5

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ABSTRACT

The sites of insertion of the transposable kanamycin-neomycin resistance-determining element, Tn5, in the *E. coli* K-12 chromosome were assessed in a collection of over 300 auxotrophs. Although mutations in at least 45 different cistrons were obtained, the distribution of insertion sites was not completely random: *proA* or *proB*; *cysG*; and *cysH*, *cysD* or *cysC* mutants were found in excess.

STRONGLY polar mutations are often due to insertion of a segment of DNA into a gene, interrupting its continuity and disrupting transcription of distal genes in the same operon (STARLINGER and SAEDLER 1976). DNA insertion elements range from small (800 to 1400 base pairs) IS elements carrying no known genes to complex phages such as Mu and λ. Intermediate in size and complexity are the transposable (Tn) elements, which carry antibiotic resistance determinants and possibly additional genes unrelated to insertion function. Mutations due to the insertion of each class of element have been obtained in *Escherichia coli* K-12 (TAYLOR 1963; JORDAN, SAEDLER and STARLINGER 1968; SHAPIRO 1969; SHIMADA, WEISBERG and GOTTESMAN 1972; KLECKNER *et al.* 1975; BERG 1977). While there is no straightforward way to select for IS element-induced insertion mutants, Tn element-induced mutants may be readily selected because of the antibiotic resistance determinants that they carry.

We have isolated over 300 *E. coli* mutants induced by the kanamycin-neomycin resistance-determining element, Tn5, to assess whether the element inserts preferentially at certain sites or randomly throughout the chromosome, and to isolate mutants for use in further studies. These insertion mutants are useful for studies of operon polarity, internal promoters, indirect suppression and null activity mutants.

MATERIALS AND METHODS

Strains: Insertion mutants were induced in *Escherichia coli* K-12 strain W3110(*thy*) using phage λ *c1857 b221* (deleted for λ *att*) carrying the transposable element, Tn5 (λ::Tn5) (D. BERG *et al.* 1975). The F′ strains were obtained from the *E. coli* Genetic Stock Center, Yale University, New Haven, Connecticut.

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Media: Tryptone broth containing 8 g/l tryptone, 5 g/l NaCl, 2 g/l maltose, and 10 mg/l thymine was used for λ::Tn5 infection of *E. coli*. The complex medium contained 5 g/l sodium citrate, 1 g/l glucose, 10 g/l tryptone, 5 g/l yeast extract, 10 mg/l thymine and 15 g/l agar. In some experiments, the complex medium was supplemented with 40 mg/l DL-glutamate, 50 mg/l DL-diaminopimelic acid, and 40 mg/l DL-alanine in order to detect cell-wall mutants. Medium E (VOGEL and BONNER 1956) plus 5 g/l glucose, 10 mg/l thymine and 2 mg/l thiamine was employed as the minimal medium. In some experiments 15 g/l sodium succinate was substituted for glucose in the minimal medium.

Mutagenesis: Cells grown overnight at 37° in tryptone broth plus 0.2% maltose were centrifuged and resuspended at about 5×10^8/ml in 0.01 M $MgSO_4$. Phage were added at a final multiplicity of infection of five to ten, and the mixture was incubated for 15 min at 30°. The mixture was then diluted ten-fold with tryptone broth lacking maltose and aerated at 30° for 30 min to allow for phenotypic expression of antibiotic resistance. The cells were spread on fresh plates containing complex agar plus 10 μg/ml neomycin sulfate (Sigma), and incubated for 48 hours at 30°. The colonies were replica plated to minimal medium, and the replicas were allowed to grow for 24 hours at 30°. Auxotrophs were purified and tested for growth requirements at 37°.

Mutant characterization: About 10^7 cells of each mutant were spread on a glucose minimal plate and tested by spotting drops of amino acid, vitamin and purine plus pyrimidine pools (individual supplements were obtained from Sigma), and also casamino acids (Difco) on each plate. After 24 to 48 hr incubation at 37°, the plates were scored for growth around or between the pools. Mutants were retested for growth on plates on which the appropriate individual components had been spotted.

After the mutants had been classified by growth requirement, they were further characterized by their ability to crossfeed or be crossfed by other members of the same phenotypic class, their ability to grow on biosynthetic intermediates and, in some cases, by F′ complementation mapping or enzyme assays.

RESULTS AND DISCUSSION

The kanamycin-neomycin resistance-determining transposable (Tn) element, Tn5, transposes from lambda to the *Escherichia coli* chromosome at a frequency of about 10^{-3}. About 1% of these antibiotic-resistant (Neo^r) isolates are auxotrophic (D. BERG 1977; this work). Since there is no evidence for multiple insertion of Tn5 during transposition (D. BERG, personal communication), the probability of obtaining a strain with two Tn elements inserted is negligible (*ca.* 10^{-6}). Therefore Tn5 is an effective single-site mutagen. Tn5 inserts into the chromosome with a higher frequency than other Tn elements (KLECKNER, ROTH and BOTSTEIN 1977), and most insertion mutants are stable, with a true revertant frequency of $\leq 10^{-6}$ (D. BERG 1977).

In this study, 305 Tn5-induced auxotrophs were isolated after infection of *E. coli* with λ::Tn5 and selected on complex medium containing neomycin. They were identified by their inability to grow when replica plated onto glucose minimal medium. Since the auxotrophs were isolated without enrichment, they reflect the natural distribution of insertion sites detectable under the conditions employed.

An accurate estimate of the reversion frequency could not be obtained for many of the auxotrophs. In some cases, the number of revertants was not proportional to the number of cells plated (competitive suppression: RYAN 1953) and in other cases indirectly suppressed revertants outnumbered true revertants.

The most conspicuous mutants of the latter type were the *proAB*::Tn5 mutants, where about 80% of the revertants were still *proAB*::Tn5. Since spontaneous *proAB* mutants are known to revert preferentially *via* forward mutation at *argD* (C. BERG and ROSSI 1974), this observation was not surprising.

Nutritional requirements of 274 of the 305 auxotrophs were determined on the basis of growth response to various metabolites. Mutants with a common requirement could be further subdivided using a combination of crossfeeding behavior, growth response on biosynthetic intermediates, F′ complementation testing and, in some cases, enzyme assays (Table 1, Figure 1). One mutant, which did not respond to any supplement on glucose minimal medium, was able to grow normally on succinate minimal medium, but was inhibited if glucose was added to the plate. Thirty mutants could not be characterized with the tests employed.

TABLE 1

Tn5-induced auxotrophs in E. coli *K-12 W3110* (thy)*

Symbol	Method of identification†	Site of insertion	Number	Total	Percent
Arg	a,b,c	B	3		
		C	4		
		E	5		
		G	4		
		H	1		
		I	2		
		unidentified	2	21	6.9
Asp		B	3	3	1.0
Cym	a	A or Ba	3		
		unidentified	1	4	1.3
Cys	a,b,c	I or J	8		
		H, D or C	53		
		E	4		
		G	22	87	28.5
Dap	e	A or E	1		
		unidentified	1	2	0.7
Gly	c	A	2	2	0.7
Gua	a,b,c	A or B	1	1	0.3
His	a,b,c	D	4		
		E	3		
		G	1		
		unidentified	4	12	3.9
Ilv	a,b,d	A	4		
		C	9		
		D	7		
		E	2	22	7.2
Leu		unidentified	16	16	5.2

TABLE 1—Continued

Symbol	Method of identification†	Site of insertion	Number	Total	Percent
Lys		A	2	2	0.7
Met	a,b,c	E	4		
		J, B, F, L or M	3		
		unidentified	7	14	4.6
Phe		A	4	4	1.3
Pro	*b*	A or B	19		
		C	2	21	6.9
Pur§	a,c	A	1		
		C, G, I	9		
		E	2		
		F	1		
		J, D, H	7	20	6.6
Pyr	a,c	A‡	6		
		B	2		
		C or D	9		
		F	1		
		unidentified	1	19	6.2
Ser	a,b,c	B	2		
		unidentified	6	8	2.6
Thr	a,b,c	A	3		
		B or C	4	7	2.3
Trp	a,f	A	2		
		B	2		
		D	2		
		unidentified	3	9	3.0
Unclassified			30	30	9.8
Glucose sens‖			1	1	0.3
Total				305	99.8

* Gene designations as in BACHMANN, LOW and TAYLOR (1976), except for the *Cym* mutants which are as in QURESHI, SMITH and KINGSMAN (1975).

† Cistron assignments were based upon: (a) growth on intermediates; (b) crossfeeding; (c) F′ complementation; (d) enzyme assays; (e) cotransduction (H. LIEBKE, personal communication); and (f) intermediate accumulation (D. BERG, personal communication).

‡ *Car*A or B.

§ Revertants accumulate in most of these strains if they are stored in broth.

‖ This mutant will grow on succinate as a carbon source, but it is inhibited by glucose.

The *argI* mutants were unexpected because wild-type *E. coli* has duplicate genes coding for isozymes of ornithine carbamoyltransferase (BACHMANN, LOW and TAYLOR 1976). Since single-step mutants defective in this enzymatic activity are not generally obtained, it appeared probable that W3110(*thy*) was either *argF* or *argI*. F′ strains carrying either *argF*$^+$ or *argI*$^+$ yielded Arg$^+$ recombi-

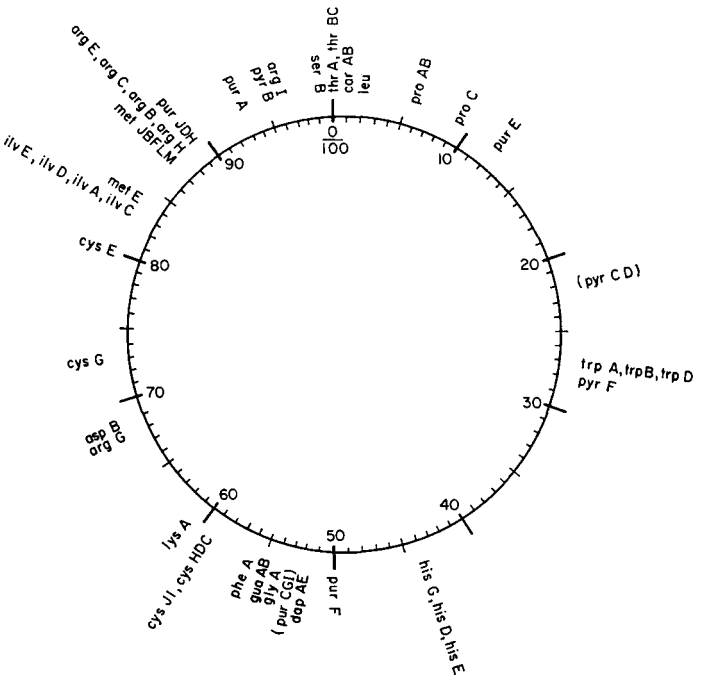

FIGURE 1.—Map locations of the mutations identified in Table 1. The map positions are according to BACHMANN, LOW and TAYLOR (1976).

nants with both Tn5-induced mutants. Since our strain of W3110(*thy*) is also *lacA* (D. BERG personal communication), we assume that this strain carries an *argF-lacA* deletion and that Tn5 is inserted into *argI*.

It is clear that Tn5 inserts at many sites in the *E. coli* chromosome (Table 1, Figure 1). However, insertion is not completely random because several classes of auxotrophs were obtained in excess. Cysteine-requiring mutants, for example, comprised 29% of the total, with two genes or gene clusters accounting for the excess: 7% of all the auxotrophs isolated were *cysG* and 17% were *cysH*, *cysD* or *cysC*. Insertion into other *cys* sites was obtained at low frequencies. *proA* or *proB* mutants were also obtained with above average frequencies (6%). A non-random distribution of Tn5 insertion sites in *lacZ vs. lacY* has been found previously (D. BERG, personal communication).

No DNA insertion element has been found to insert into the bacterial chromosome randomly. λ (deleted for *att*) inserts preferentially into *pro* in *E. coli*, but also inserts into other genes at <3% of the *pro* frequency (SHIMADA, WEISBERG and GOTTESMAN 1973). Mu induces an excess of *pur*, *pro* and *met* mutants in *E. coli* (TAYLOR 1963). The relative frequencies of Tn*10*-induced auxotrophs in *S. typhimurium* differ from one report to another (KLECKNER *et al.* 1975; KLECKNER, ROTH and BOTSTEIN 1977). The distribution of phenotypic classes in a small sample of Tn5-induced auxotrophs obtained in a previous study with *E. coli* (KLECKNER, ROTH and BOTSTEIN 1977) differs from our findings (Table 1), but

the major discrepancy between the two sets of data (the absence of *pro* mutants in that study) is due to the fact that a *pro* auxotroph had been used as the recipient for Tn*5* transposition (H. LIEBKE, personal communication).

The patterns of insertion of Tn*5*, and Tn*10* are different, but comparable, if mutants are classified only with respect to required metabolite. However, when mutants within a gene are mapped, the Tn*5* and Tn*10* patterns are dissimilar. For example, in the *lacZ* gene of *E. coli*, only 5% of the Tn*5* insertions are not separable by recombination (D. BERG 1977), while 86% of the Tn*10* insertions fall in one small region (FOSTER 1977). Insertion of Tn*5* seems, therefore, more random than insertion of *Tn10*.

Transposable elements carrying drug-resistance determinants provide a simple direct selection procedure for the isolation of viable insertion mutants without recourse to indirect selection procedures or concern about the isolation of multiple mutations. In addition, since the transposition of a Tn element into a gene eliminates all detectable gene function and has a polar effect upon the expression of operator-distal genes (D. BERG 1977; KLECKNER *et al.* 1975; HEFFRON, RUBENS and FALKOW 1977), insertion mutants can be used in studies in which the absence of a gene product is essential and in the detection of internal promoters. Using crossfeeding, we have been able to detect the internal promoters previously described in the *argCBH* (ELSEVIERS *et al.* 1972; JACOBY 1972), and *his* (ATKINS and LOPER 1970) operons, as well as a previously overlooked internal promoter in the *ilvGEDA* operon (C. BERG *et al.*, 1979). Additionally, the mutants isolated will be invaluable in the study of indirect suppression because true revertants, which lose Tn*5* (D. BERG 1977), may be readily distinguished from indirectly suppressed revertants, which retain Tn*5* (unpublished), on the basis of antibiotic resistance.

Since transposable elements insert into a large number of different sites, many mutations which were previously difficult to select now may be manipulated easily by transduction or conjugation because of linkage of the mutation to the antibiotic-resistance phenotype. In addition, since the possible sites of insertion differ from element to element, it is useful to have several well-characterized collections of mutants induced by the insertion of different transposable elements.

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