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Fusion of the *Escherichia coli lac* Genes to the *ara* Promoter: A General Technique Using Bacteriophage Mu-1 Insertions

(*ara*, *lac*, *trp* operons/gene regulation/ϕ80 transducing phage/nonhomologous recombination)

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ABSTRACT **The *lac* genes were fused to the *ara* promoter by means of phage ϕ80 translocations of the *lac* and *ara* genes to *att80*. Homology for a crossover between the nonhomologous *ara* and *lac* operons was provided by mu insertions. Selection for recombinants within the mu insertions generated strains that had the *ara* promoter on one side of a mu insertion and the *lac* genes on the other side. *ara–lac* fusions were obtained from these strains by deleting the mu insertion. These fusions extend the techniques available for studies on the *lac* operon to studies on the *ara* operon. It should be possible to fuse other operons by this method.**

One of the best characterized genetic regulatory systems is the *lac* operon of *Escherichia coli*. A major reason that analysis of the *lac* operon has progressed so rapidly is that there exists a very sensitive and easy enzyme assay (for β-galactosidase) and a wide variety of useful lactose analogues (1). These galactoside analogues have provided a range of selective and screening techniques not available in most other regulatory systems. It is possible to extend the techniques available for the *lac* operon to other genetic systems by genetically fusing the *lac* structural genes to the controlling elements of the genes of interest (2–4). For instance, the existence of fusions of the *lac* operon to the *trp* operon has allowed the isolation of the *trp* repressor and additional genetic analysis of *trp* regulation (5,6).

The fusion of the promoter of one operon to the structural genes of another operon requires the physical joining of the DNA of the two operons. The joining can be obtained as a recombination event between the two operons. However, since two different operons have different base sequences, recombination between them is expected to be very rare. One approach for obtaining recombination between two operons with different base sequences is to first insert separately into each one, at the positions where recombination is desired, identical pieces of DNA. The two operons with identical inserted pieces of DNA can then undergo recombination within the inserted sequences. The result of one such recombination event will be a recombinant insertion piece of DNA which has the promoter end of one operon on one side and the terminal end of the second operon on the other side. Subsequent deletion of the recombinant insertion piece of DNA will yield a fusion of the two operons.

We have used this technique to fuse the *lac* structural genes to the promoter of the *ara* operon. Bacterial strains carrying these fusions express the *lac* genes under the regulation of the *ara* promoter. The identical insertions in the *ara* and *lac* operons needed for the fusions were insertions of bacteriophage mu. Mu inserts its genome upon lysogenization into random sites on the *E. coli* chromosome (7).

MATERIALS AND METHODS

Chemical and Media. Media are described elsewhere (9, 10). Minimal media contain each stated sugar at 0.2% and amino acids at 20 μg/ml. Nalidixic acid (Schwarz/Mann) was added at 20 μg/ml; spectinomycin SO_4 (a generous gift of the Upjohn Co.), at 200 μg/ml; streptomycin, at 114 μg/ml; isopropyl-β-D-thiogalactoside (Schwarz/Mann), at 1 μg/ml; 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG, Cyclo Chemical Corp.), at 40 μg/ml; and sodium citrate, at 5 μg/ml.

Phage and Bacteria. Bacterial strains are listed in Table 1. Bacterial and phage techniques have been described (10).

TABLE 1. *Strains*

D7048:	HfrCav, *thr*, *ara*Δ(*DABIOC*) *714*, *val*^{R}, *trp*, *met*.
D7053:	HfrCav, *thr*, *araD139*, *trp*, *met*.
EF735:	F'KLF-1, *thr*$^+$, *ara*Δ(*AB*) *735*, *leu*$^+$/F$^-$ *araD139*, Δ(*araABC*, *leu*) *7697*, *nalA*, *recA*, *spcA*, *strA*.
M8820:	F$^-$, *araD139*, Δ(*araABIOC*, *leu*) *7697*, Δ(*proAB*, *lac*) *XIII*, *strA*.
M8834m:	F$^-$, Δ(*araOC*, *leu*) *1109*, *strA*, *mal*(λv^R):mu(cts).
X7740:	F$^-$, *araC(J-3)*, Δ(*proAB*, *lac*) *XIII*, *galE*, ϕ80dII*lac-AYZ* Δ(*lacOPI*, *tonB*, *trpA*) *W209*, *nal*, *spcA*, *strA*.
XA8810:	F$^-$, *araD139*, Δ(*araABIOC*, *leu*) *7697*, ϕ80dII*ara-DABIOC*, *strA*.
XA8811:	F$^-$, *araD139*, Δ(*araABIOC*, *leu*) *7697*, ϕ80dII*ara-DABIOC* Δ(*attPB'*, *tonB*, *trp*)*3*, *strA*.

These strains were constructed from strains in the collection of J. Beckwith. All the *ara* regions mentioned, except *araC(J-3)*, originated in *E. coli* B/r strains obtained from E. Englesberg. All strains used were *E. coli* K-12.

Abbreviations and notation (8, 9): *araC*, *araOIBAD*, *galKTE*, *lacI*, *lacPOZYA*, and *mal* are operons for the utilization of the sugars L-arabinose, galactose, lactose, and maltose. *leu*, *met*, *pro*, *thr*, and *trpPEDCBA* are operons for the biosynthesis of leucine, methionine, proline, threonine, and tryptophan. *araOI*, *lacPO*, and *trpP* denote promoter and regulatory sites; other letters represent structural genes. *araC* and *lacI* are regulatory genes. *tonB* is a genetic locus necessary for the adsorption of various phages and colicins. *att80* is the chromosomal attachment site for bacteriophage ϕ80. *nal*, *spc*, *str*, and *val* are genes determining sensitivity and resistance to nalidixic acid, spectinomycin, streptomycin, and L-valine. ϕ80d denotes a defective ϕ80 transducing phage carrying the stated genetic region, as ϕ80d*ara*. XG, 5-bromo-4-chloro-3-indolyl-β-D-galactoside.

Mu lysates were grown from lysogens obtained from A. Bukhari and M. Howe (11). The mu phage used in all experiments contained a temperature-sensitive repressor (*cts*).

Method of Mu Lysogeny. A stationary phase culture (0.1 ml) was plated with 2.5 ml of H top soft agar on a TYE (tryptone-yeast extract) plate. Drops of a mu (*cts*) (11) lysate were placed on top and the plate was incubated overnight at 30°. Survivors from the center of the spots were picked and patched onto the appropriate selective plate. Colonies that grew up were picked and streaked to single colonies. Only one mu lysogen from each patch was used in order to have all independent lysogenization events. The purified colonies were checked for their appropriate phenotypes. Mu lysogeny was checked by streaking across a streak of a mu-sensitive strain on a TYE plate at 42°. A mu (*cts*) lysogen has few survivors at 42° and releases phage which kill nearby mu-sensitive cells. The mu prophage was shown to be linked to the site of the inactivated gene by transducing back the wild-type gene with P1. If the mu prophage was inserted in the inactivated gene, and if there was no other mu prophage, then such a transductant would have lost the mu prophage. (The frequency of mu lysogens that had prophages not linked to the inactivated gene varied considerably in different experiments.)

Mu Insertions in araB. Mu was first inserted into the *ara* genes of the *araD*$^-$ Hfr strain D7053. Growth of D7053 is inhibited in the presence of L-arabinose, since it accumulates the degradation product L-ribulose 5-phosphate (13). Mu lysogens of D7053 that have insertions in the *ara*C, *B*, or *A* genes were selected as lysogens that formed *ara*$^-$ colonies on tetrazolium plates containing L-arabinose.

To tell which *ara* gene (*A*, *B*, or *C*) the mu prophage was in, the following two tests were done. (*i*) The mu lysogens were streaked on minimal glycerol plates containing citrate, threonine, methionine, and tryptophan with and without arabinose. Only *araA*$^-$ *B*$^+$*C*$^+$ cells are inhibited from growing on the minimal plates with arabinose (14). (*ii*) The mu-lysogens were mated with M8834m on minimal arabinose plates at 30°. Only the *araA*$^-$ and *B*$^-$ lysogens could give rise to *ara*$^+$ recombinants with the *araC* deletion (15) in M8834m. By means of these two tests, the first six mu lysogens picked were shown to consist of two insertions in *araA*, three in *araB*, and one in *araC*.

Next, the mu insertions in the *araB* gene of D7053 were recombined into the ϕ80 translocated *araB* gene of XA8810 (Fig. 1A). A derivative of XA8810, XA8811, was used for this purpose. XA8811 has a *tonB-trp* deletion (9) which extends into one end of the ϕ80dII*ara* prophage and prevents curing. This deletion does not affect the *ara* genes. The insertions in the *araB* gene of D7053 were crossed into XA8811 by cross streak matings on minimal glucose–citrate–tryptophan–streptomycin plates. *leu*$^+$*ara*$^+$*str*^{R}*tonB*$^-$*trp*$^-$ recombinants were picked. These recombinants are diploid for the *ara* genes. At the normal *E. coli ara* locus near *leu* they have a mu insertion in *araB* and the *araD139* mutation. At the transposed *ara* locus at *att80* they have the normal *ara* genes. Derivatives of these strains that have the *araB* mu insertions recombined into both *araB* genes were found as *ara*$^-$ colonies on arabinose McConkey indicator plates at a frequency of about 10^{-4}.

The final step in the construction of strains of type A was to replace the deleted part of the ϕ80 dII*ara* prophage. This was done by transducing the two homogenotes to *trp*$^+$*tonB*$^+$*ara*$^-$ with P1 grown on XA8810.

lac$^-$*Mu Insertions in trp Genes.* In strain X7740 the structural genes of the *lac* operon are under the control of the *trp* promoter (Fig. 1B) (3). *lac*$^-$ derivatives of X7740, which is *galE*$^-$, can be obtained as cells that grow in the presence of lactose (16). (Lactose is transported and cleaved to yield galactose. The galactose is then converted by the *galK* and *T* gene products to intermediates that stop cell growth when they accumulate in the absence of the *galE* gene product.) Mu insertions in the *trp*, *lac*, or *gal* genes of X7740 were obtained as mu lysogens that grow on glycerol–lactose–proline–citrate–tryptophan plates. Mu insertions in the *trp* genes of X7740 are *lac*$^-$ because they separate the *lac* genes from the *trp* promoter. Mu insertions in *trpB*, *C*, *D*, or *E* were distinguished from other mu insertions by their inability to use indole in place of tryptophan. [Only the *trpB* gene product is required for the synthesis of tryptophan from indole (17). Mu insertions in *trpB* inactivate the gene and mu insertions in *trpC*, *D*, or *E* block the expression of the *trpB* gene because they separate it from the *trp* promoter.] About 10% of the X7740 mu lysogens obtained on plates containing lactose could not use indole in place of tryptophan for growth. Verification that the mu insertions were in the *trp* region was done by transducing them with P1 to *trp*$^+$ and scoring for the loss of mu lysogeny.

The locations of the mu insertions in the *trp* genes were determined further as follows: (*i*) Insertions in *trpB* were distinguished from insertions in *trpE*, *D*, and *C* because they excrete into the media the tryptophan biosynthetic intermediate indole-glycerol 3-phosphate (12). (*ii*) Insertions in *trpE* and most of *trpD* were distinguished from other *trp* insertions by their low expression of the *lacZ* gene, which comes from the low level internal *trp* promoter in *trpD* (18). This low level expression was checked for five *trp* insertions by streaking them on minimal glucose plates containing proline, tryptophan, citrate, and the sensitive β-galactoside indicator XG (1). The indicator XG is colorless until it is cleaved by β-galactosidase to yield a blue color. On these plates colonies of two of the five mu insertions checked were light blue, indicating the transcription from the low level internal *trp* promoter in *trpD* was not blocked. Since these two insertions also did not excrete indole-glycerol phosphate, they are probably located in *trpE* or *D*. Colonies of the other three insertions were almost as white as the colonies of a control *lac* deletion strain. (It is not known where this very low level of β-galactosidase is promoted.) Since these cells excreted indole-glycerol phosphate and could not utilize indole in place of tryptophan for growth, their mu insertions are probably in *trpB*.

After the mu insertions in *trp*E, *D*, *C*, or *B* in X7740 were obtained, spontaneous mutants capable of growth in the presence of galactose (*galK*$^-$ or *gal*$^-$ polar on *galK*) were selected on glycerol–galactose–citrate–tryptophan–proline plates.

Deletions of Mu Insertions. Cells containing the recombinant mu (*cts*) insertions (Fig. 1C) were grown to stationary phase at 30° in LB broth and plated at 42° with 2.5 ml of soft agar on minimal arabinose–proline–XG plates. At 42° the mutant mu repressor is inactivated, causing the mu lysogen to be induced and kill the cell. Cells that have deletions of the mu lysogen can survive to form colonies (19). After 1 day the plates were shifted to 37° for better growth of the colonies. Fewer than 10^8 cells were plated per plate to minimize the killing of the mu-deleted cells by the mu phage released on the plate from

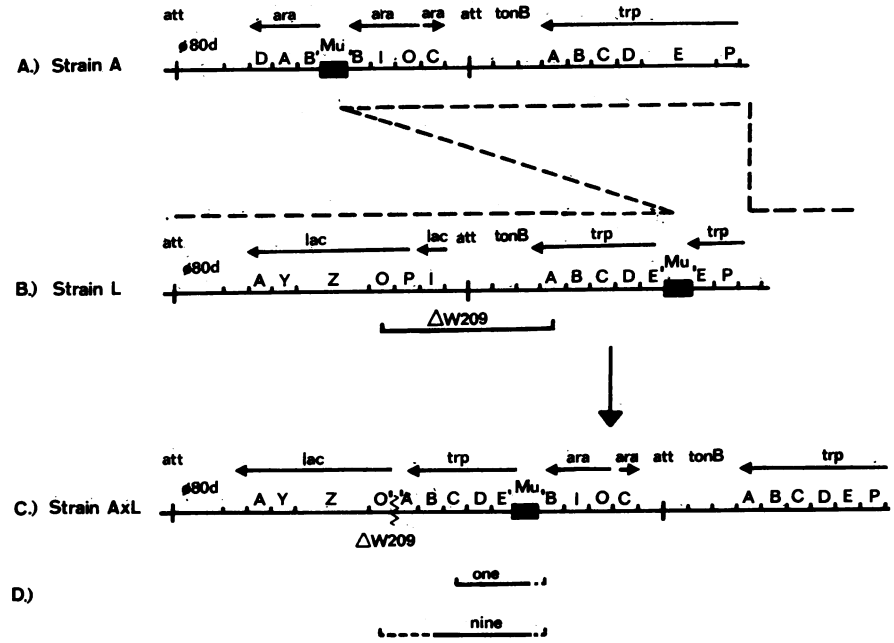

FIG. 1. A cross between a type A and a type L strain. A prime next to a letter indicates that the genetic region denoted by the letter is deleted or interrupted on the side that the prime is written, as 'B. The genetic regions are not drawn to scale. The mu insertions, denoted by thick solid bars, are very much larger than indicated. (A) The relevant regions of the chromosome of the type A strain. The mu insertion in *araB* has been recombined onto both *ara* regions. The *ara* region near *leu* is not shown. (B) The chromosome of the type L strain with a mu insertion in *trpE*. The region deleted by ΔW209 is not present and is drawn only to show how the deletion was derived. ΔW209 fuses the *lacZYA* genes to the *trpP* promoter. The mu insertion blocks transcription of the *lac* genes from the *trp* promoter. (C) A recombinant of the type L strain which has received a segment of the chromosome of the type A strain. The sequence of genes in the recombinant is indicated by the broken line in (A) and (B). The region deleted by W209 is not drawn. (D) The position of 10 deletions that fuse the *lacZYA* genes to the *araOI* control region. Broken lines denote the undetermined extents of the deletions.

the other cells. (When more than 10^8 cells were plated the frequency of survivors went down from 10^{-5} to 10^{-8}.)

RESULTS AND DISCUSSION

General Outline. Two strains that have the *ara* and *lac* operons transposed to the same region of the *E. coli* chromosome were lysogenized with bacteriophage mu. In one strain (type A) the mu prophage was inserted in *araB*. In the other strain (type L) the mu prophage was inserted between the *lacZ* gene and its promoter. These two strains were then crossed and a recombinant with a crossover between the two mu insertions was obtained. The *ara* promoter was on one side of the recombinant mu insertion and the *lac* genes were on the other side. Finally, the mu insertion was deleted to fuse the two operons.

Strain Type A. The starting strain for constructing strain A was XA8810. The transposition of the *ara* operon to *att80* was obtained as a lysogen of the defective *ara* transducing phage φ80dII*ara* (Fig. 1A) (9; M. Biales, S. Gottesman, and J. R. Beckwith, personal communication). The direction of transcription of the *ara* operon in this lysogen is the same as the direction of transcription of the nearby *trp* operon.

Strain type A was constructed from XA8810 by inserting mu into the *araB* gene. This was not done directly. The mu insertion was first obtained in the *araB* gene of strain D7053. The insertion was then recombined into the *araB* gene of XA8810. The details are described in *Methods*.

A mu insertion in *araB* was chosen because it was close to the promoter for the *araBAD* operon. All of the regulatory sites of the *araBAD* operon are intact on one side of the insertion. No complete gene is known to exist between the mu insertion and the *ara* promoter.

Strain Type L. The starting strain for constructing strains of type L was X7740. X7740 has the *lac* genes transposed to *att80*. This was originally obtained as a lysogen of φ80dIIlac (4). The direction of transcription of the *lac* operon is the same as the direction of transcription of the *ara* operon in type A strain. The requirement for the mu-insertion in a type L strain is that it be between the *lacZ* gene and its promoter. Since the wild-type *lac* operon on φ80dIIlac has a very short space (20) between the promoter and the Z gene in which to isolate a randomly occurring mu insertion, we decided to use the *trp–lac* fused operon W209 (3) (Fig. 1B). This operon has almost the full length of the *trp* operon between the *lacZ* gene and its promoter, which in this operon is the *trp* promoter. The wild-type *lac* promoter is no longer present in this fusion. Thus strains of type L were obtained from X7740 as *lac*$^-$ lysogens of mu that had the mu prophage inserted in the *trp* region. Such an insertion is *lac*$^-$ because it separates the *lac* genes from their promoter. The details are described in *Methods*.

The Cross. The next step in obtaining the *ara–lac* fusion was to cross the type A and L strains and to obtain a crossover within the two mu insertions. The cross is shown in Fig. 1. One type A strain, called A3, was crossed with five type L strains. Several strains of type L were used because only 50% of them were expected to have mu inserted in the same orientation as the mu insertion of strain A3 (11). Strain A3 was made into a male donor by introducing the F'KLF1 episome (21) in a mating with EF735. Strain A3 could then donate the *att80-trp* region of its chromosome to the type L strains by the process of chromosome mobilization (22). The recombinants with a crossover within the mu insertions had received the

TABLE 2. *β-Galactosidase assays of fusion strains*

Fusion strain	Averaged number of independent assays	Enzyme units without arabinose	Enzyme units with arabinose	Induction ratio
HI	3	4.2	2400	570
FI	3	4.4	1900	440
H3	1	2.8	1400	520
F4	3	0.94	720	770
H2	1	0.76	640	850
L2	1	0.52	350	680
L1	3	0.55	280	510
L3	1	0.48	175	370

The cells were grown in minimal glycerol-proline (0.1%) medium with and without arabinose and assayed as described (10).

trp$^+$ genes to the right of the mu insertion in strain A3 and had kept the *lacZ* gene on the left of the mu insertion in a type L strain. These were selected as *trp*$^+$*nal*^{R}*spc*^{R} very light blue colonies on minimal glucose–XG–isopropylthiogalactoside–proline–streptomycin–nalidixic acid–spectinomycin plates. The very light blue color of the colonies indicates the presence of a very low level of β-galactosidase which is made from a *lacZ* gene with no high level promoter, as described in methods. *trp*$^+$*nal*^{R}*spc*^{R} recombinants that did not have a crossover within the mu insertion had to have a crossover to the left of the *lac* genes of the type L strain. Such recombinants would form either white colonies because they had no *lacZ* gene, or dark blue colonies if they had crossed in the wild-type *lac* region of A3.

Crossovers within the mu insertions were obtained for four out of five of the type L strains used in the crosses with A3. About 5% of the *trp*$^+$ recombinants had a crossover within the mu insertions when the mu insertions had the same orientation. (One of the type L strains apparently had a mu insertion in an opposite orientation to the insertion in A3.)

Deletions of the Recombinant Mu Insertion. The final step in obtaining the fusions was to delete the mu (*cts*) insertion which was between the *ara* promoter and the *lac* genes (Fig. 1C). Deletions were selected on minimal arabinose–proline–XG plates at 42°. The details are described in *Methods*. Cells with deletions of the mu insertion that fused the *lac* genes to the *ara* promoter would form dark blue colonies. Other deletions would form white or light blue colonies. The dark blue colonies appeared at a frequency of 10^{-2}–10^{-3}. All the colonies that had been dark blue on arabinose–proline–XG plates formed very light blue colonies on glucose–proline–XG plates without arabinose. This indicated that the *lac* genes were induced by arabinose.

Assays. Strains with these putative fusions of the *lac* genes to the *ara* promoter were assayed for β-galactosidase (the *lacZ* gene product) with and without arabinose (Table 2). In order to avoid the complicating effect of the utilization of the arabinose as a carbon source, the strains containing the fusions were first made *ara*$^-$ by crossing in, at the normal *ara* chromosome locus near *leu*, the *ara* (*CBAD*) *714* deletion of D7048. These *ara*$^-$ derivatives of the fusion strains grow on minimal lactose plates only when arabinose is also present.

All the fusions tested were induced by arabinose with about the same induction ratio. However, the absolute levels of expression of the *lac* genes were different. These differences may be due to polarity effects (2, 23, 24) at the site of the deletion of the mu insertion.

Mapping. Since the prophage between the *ara* promoter and the *lac* genes (Fig. 1C) was deleted to yield the fusions, mapping experiments were carried out to try to determine if all of the mu prophage had been removed and how much, if any, of the *ara* and *trp* genetic material had also been removed.

To map the remaining *ara* and *trp* genetic material, the fusions were first incorporated onto bacteriophage φ80. Since the left side of the fusion came from φ80d*lac* and the right side from φ80d*ara*, there was a φ80 attachment site on each side of the fusions (Fig. 1C). φ80 defective transducing phage carrying the fusions were obtained by ultraviolet light induction of the defective φ80 prophage of the strains carrying the fusions followed by adsorption of helper φ80 at a multiplicity of infection of 1.0. It was not known whether the length of DNA between the φ80 attachment sites was the correct length for packaging in φ80, but for all 16 of the fusions used some transduction of the *ara–lac* fusions was obtained. The selection in spot transductions was for *lac*$^+$ on minimal–lactose–arabinose–leucine–proline (0.02%) plates, using strain M8820. *lac*$^+$ transductants of strain M8820, which almost always contained lysogens for the wild-type φ80 helper as well as for the defective phage, were picked and purified. They were induced with ultraviolet light to give high titer (about 10^8 transductants per ml) lysates of each fusion. The *ara* genes of the φ80d*ara–lac* fusion phage were mapped by spot transduction with strains containing various *araA*, *B*, and *C* point mutations and deletions. The selection was for growth on minimal arabinose plates. The 16 independent fusions tested all complemented with *araC*$^-$ mutations and recombined with the *araB14* mutation (15) which maps closest to the site of the mu insertion in *araB*. Thus, none of the fusions tested were deleted into the *araB* gene past the site of the *araB14* mutation. They may in fact have left some of the mu lysogen nearest to the *araB* gene. The *trp* genetic material in the fusions was mapped by spot transducing strains containing various *trp* point mutations on minimal arabinose plates without tryptophan. A low background frequency of transduction (few colonies per spot) was obtained for all the lysates, including a control lysate containing only the φ80 helper phage. This occurs because the *trp* genes are very near the φ80 attachment site and φ80-*trp* transducing phage are readily formed (23). High frequency transduction (confluent growth under the spot) was obtained for only one of the φ80d-*ara–lac* phages tested. This phage transduced only *trpB* point mutations at a high frequency. Since the mu insertion in *trp* from which this fusion was obtained was in *trpD* or *E*, there was between the mu insertion and the *lac* genes the *trpC* and *trpB* gene. Since this fusion transduced only *trpB* mutations at a high frequency, it is probably deleted for the *trpC* gene.

To show that the *trpB* high frequency transductants from this phage were due to an *ara–trp–lac* fusion and not to a new φ80 *trpB* transducing phage, several of the *trpB* transductants, obtained on minimal arabinose plates, were streaked to single colonies and tested for their tryptophan requirement on minimal arabinose and glucose plates. They were *trp*$^+$ only on the arabinose plate, implying that the *trpB* gene product was induced by arabinose. On the glucose plate *trp*$^+$ recombinants

were seen at a high frequency. None of the other 15 fusions obtained from the same mu insertion in *trpD* or *trpE* transduced either *trpB* or *C* mutations at a high frequency. Thus, their deletions of the mu insertion must have extended at least into *trpB*.

Mu genetic material that might remain in the fusions was tested for by looking for recombination with mu nonsense mutations (11). Six mu lysates with nonsense mutations in different mu genes were spotted on lawns of M8820 lysogens of 16 ϕ80d*ara–lac* fusions. No recombinant plaques were observed with any of the mu nonsense mutations. Included were nonsense mutations in the two mu terminal genes *A* and *S*.

A summary of the mapping results for 10 fusions is shown in Fig. 1D. All 10 of these fusions were isolated from the same recombinant between A3 and a type L strain with mu inserted in *trpD* or *E*.

In conclusion, a technique is described for placing the *lac* structural genes under the control of the *ara* promoter. Similar techniques can be used to fuse other genes and promoters in *E. coli*.

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