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Use of *argA-lac* Fusions to Generate Lambda *argA-lac* Bacteriophages and to Determine the Direction of *argA* Transcription in *Escherichia coli*

THOMAS ECKHARDT

Department of Microbiology, New York University School of Medicine, New York, New York 10016

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Fusions of *lac* genes to the *argA* operator were constructed, and λ phages carrying these fusions were isolated and characterized. With the aid of a λ phage carrying an *argA-lac* fusion, the direction of *argA* transcription on the *Escherichia coli* chromosome was determined to be clockwise.

In the course of work on the regulation of arginine biosynthesis in *Escherichia coli*, I became interested in isolating lambda transducing phages in which the structural genes of the *lac* operon (*Z* and *Y*) are fused to the control region of arginine genes. In such fused genes, the formation of β-galactosidase may be under the control of arginine. Because of the stability of this enzyme and the ease with which it can be measured, studies on the control of arginine biosynthesis are facilitated.

In the present paper I describe the isolation of such lambda transducing phages by the Casadaban (2) method. These phages were used to determine the direction of *argA* transcription on the *E. coli* chromosome.

MATERIALS AND METHODS

Media and strains. The media used here have been described elsewhere (2, 10). Yeast extract-tryptone (YT) medium supplemented with thymine (200 μl/ml) and 2 mM $CaCl_2$ was routinely used. X-gal medium (20 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside [X-gal] per ml in minimal medium containing 0.1% glucose) with either *N*-acetyl-L-ornithine (50 μg/ml) or L-arginine hydrochloride (100 μg/ml) was used for identification of the *argA-lac* fusions. Bacterial strains and phages are listed in Table 1.

Chemicals. X-gal was from Bachem Inc., Marina del Rey, Calif.; mitomycin and *N*-acetyl-L-ornithine were from Sigma Chemical Co., St. Louis, Mo.; and chloramphenicol was from Calbiochem, La Jolla, Calif.

Enzyme assays. β-Galactosidase was assayed by the method of Miller (10). Ornithine transcarbamylase was measured as described by Jones et al. (7). Enzyme assays were performed in toluenized cells. Protein concentrations were determined by the Miller method (10). Specific enzymatic activity is expressed as micromoles of product formed per minute per milligram of protein under standard conditions.

Genetic techniques. (i) Phage Mu *cts* lysogeny. Standard techniques were used to obtain Mu *cts* lysogens (1). Enrichment of the Mu *cts* lysogens for arginine auxotrophs with ampicillin yielded about 1% arginine auxotrophs. *argA* insertion mutants were identified by their ability to grow on *N*-acetyl-L-ornithine and not to be restored to the Arg^+ phenotype when lysogenized by a lambda phage carrying the *argECBH* cluster. To verify that the *argA* mutations were due to Mu *cts* insertion and that they were single lysogens for phage Mu *cts*, the mutants were transduced to the Arg^+ phenotype with phage 363. Single Mu *cts* insertions into the *argA* gene thereby became heat resistant because of the loss of the heat-inducible Mu prophage. A similar procedure, using either phage 363 or a suitable Hfr strain, allowed verification of λp1(209) or λp123(209) integration into the Mu prophage.

For isolation of the fusion strains, the procedure of Casadaban (2), slightly modified, was followed.

(ii) Lambda lysogeny. The lambda phages used lack the lambda attachment site and lysogenize stably only with a low frequency. To obtain a better selection for stable lambda lysogens, the lambda phages [either λp1(209) or λp123(209)] were spotted together with λcI(*int*)9*h*80 (10^8 plaque-forming units of each phage type) on a lawn of the desired Mu lysogen (10^8 bacteria in 2.5 ml of soft agar layered on YT plates). The survivors appeared as turbid areas within the lysis spot. A small piece of this turbid area containing the lambda lysogens was picked and streaked out for single colonies on YT plates seeded with 10^8 λcI(*int*)9*h*80. Lambda lysogens, which could be recognized as round, nonmottled colonies, were picked and patched onto a master plate and incubated overnight at 32°C. The lysogens were tested for stable integration of the lambda phage by replicating the master plate onto a YT plate seeded with a lambda-sensitive strain (MC4100). These patches were then induced for phage lambda by exposing the plates to ultraviolet light. Patches of the unstable lambda lysogens gave an area of complete lysis, whereas the stable lysogens formed only a thin halo of lysis around the patch.

Deletion of phage Mu to yield *lac* fusions. An

TABLE 1. *Strains used*

Strain	Genotype[a]	Source
E. coli		
MC4100	F⁻ *araD139 ΔlacU169 rpsL thi*	M. Casadaban (2)
4100A	MC4100 with *argA*::−Mu *cts*	
MA5	F⁻ *argA*	W. K. Maas
EC113	F⁻ Δ(*lac-pro*) *argD argR aroE rpsL*	Own collection
EC135	EC113, but *argR*⁺	Own collection
EC90	EC113, *thyA* (spontaneous) lysogen for λAZ-7 in the *argA* region	Own collection
EC91	EC90, but *thyA*⁺ *argA* (*argA* from MA5)	Own collection
EC92	EC91, spontaneous *thyA*	Own collection
EC93	EC90, but *lacY* on λAZ-7 (NG mutagenesis)	Own collection
EC94	EC93, but *thyA*⁺ *argA* (*argA* from MA5)	Own collection
EC95	EC94 spontaneous *thyA*	Own collection
Phage		
λp1(209)	λpcI⁺ *lacA?YZO'* −Δ*W209* −*trp'AB'*::+Mu	M. Casadaban (2)
λcI(*int*)Δ9*h*80		Shimada (11)
λAZ-1, λAZ-7	λcI⁺ *lacA?YZO'?* −Δ*W209?* −*trp'AB'?* −*arg'AO*	This work
Mu *cts*		M. Howe
363		W. K. Maas (3)

[a] NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Mu terminology: ::, insertion of Mu into a given gene; (+) and (−), directions of insertion into the gene (5); prime ('), deletion of a part of a gene on the side indicated by the prime.

overnight culture of the lambda-Mu lysogen in YT medium (40 ml) was centrifuged, suspended in 5 ml of minimal medium, and incubated for 30 min at 44°C to induce the Mu prophage. Portions of 0.5 ml were then mixed with 3 ml of soft agar (0.6% agar in water) and poured onto prewarmed minimal medium plates containing 0.5% lactose and *N*-acetyl-L-ornithine (50 μg/ml). After the top agar solidified, the plates were incubated overnight at 42°C and then grown for an additional 24 h at 37°C. Colonies were picked, patched onto minimal medium plates containing glucose (0.5%) and L-arginine hydrochloride (100 μg/ml), and incubated overnight at 37°C. The master plate was replicated onto X-gal medium supplemented with either 100 μg of L-arginine hydrochloride per ml (repressed conditions) or 50 μg of *N*-acetyl-L-ornithine per ml (partially derepressed conditions) and incubated for 3 h at 37°C to allow derepression. The plates were sprayed heavily with chloramphenicol (2.5 mg/ml) to stop cell growth and incubated at 32°C, and blue color formation was then monitored. By this method it is possible to detect small increases in β-galactosidase activity due to derepression. Fusions to an operator with even a small repression coefficient are easily recognizable.

Preparation of lambda phages carrying *arg-lac* fusions. The *arg-lac* fusion strains were induced for phage lambda by adding mitomycin C (1 μg/ml) to an exponentially growing culture (10^8 bacteria per ml) in YT medium. The released lambda phages were scored for β-galactosidase production on X-gal medium, with strain MC 4100 as the recipient. Lambda phages that transduce *lacZ* form blue plaques. This first induction gave a heterogeneous phage population. Stable homogeneous phage lines were obtained by picking single plaques and growing the phage by infection. Some of the lambda phages carrying *arg-lac* fusions turned out to be sensitive to detergent and antifoam agents.

Transduction with phage 363. For transduction with phage 363, standard procedures were followed (10), except when lambda lysogens were used as recipients. Starvation may cause induction of a lambda phage, especially starvation for thymine, thereby reducing the number of transductants. To avoid such induction, cells were washed after transduction with a mixture of YT medium (without Ca^{2+}) and minimal medium (1×-strength final concentration), to remove excess phage and calcium. The cells were resuspended in the same medium and grown for 2 h to allow expression of the transduced marker and avoid starvation on selective plates.

Other general techniques were performed by the Miller method (10).

RESULTS

Isolation of *argA-lac* fusions strains. To isolate strains in which the *lac* genes were fused to the *argA* operator, a heat-inducible phage Mu (*cts*) was first inserted into the *argA* gene of strain MC4100 (strain 4100A). In a second step, a λpcI⁺ phage carrying the *lac* genes, part of the *trp* operon, and part of phage Mu was integrated into the inserted Mu prophage (2). This lambda phage lacks the original attachment site and, in the absence of the chromosomal *lac* gene, only lysogenizes stably in a Mu lysogen by using its Mu homology for integration (Fig. 1a). This puts the *lac* genes close to the *argA*

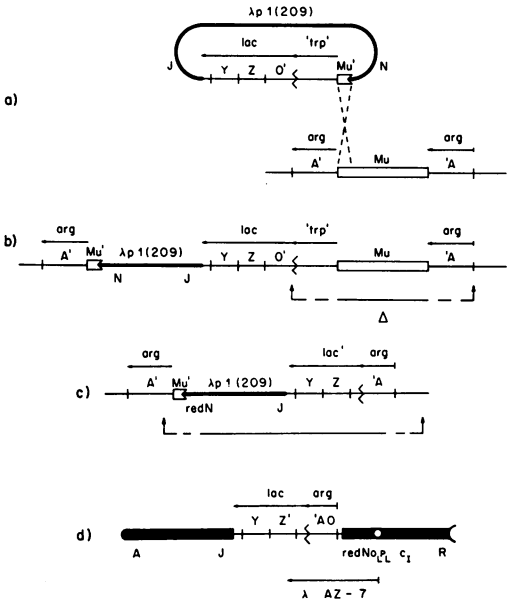


FIG. 1. *Construction and structure of argA-lac fusions and the structure of lambda phages carrying an argA-lac fusion. (a) Lysogenization of an argA::Mu cts lysogen with λp1(209) or λp123(209). (b) Structure of the λ-Mu lysogen. The dotted line indicates variations in the end points of the deletions upon induction of Mu prophage. (c) Structure of an argA-lac fusion. The dotted line indicates variation in the excision of phage lambda yielding lambda phages carrying different amount of chromosomal DNA. (d) Structure of a lambda phage carrying an argA-lac fusion (λAZ-7). Symbols: ——, bacterial chromosome; ←—|, direction of transcription with starting point (promotor); ≷, fusion point; ▭, Mu phage; ▬, lambda phage (cI, N, J, R, and red are λ genes; o_L and p_L are the left-hand operator and promotor, respectively, of phage lambda).*

operator. Because of the absence of the *lac* promoter, the *lac* genes are not expressed. Since the direction of the *argA* gene is not known, it was necessary to insert the *lac* genes in both directions. This was achieved by using two different lambda phages carrying either the *c* end of phage Mu [λp1(209)] or the *S* end [λp123(209)]. Because the *lac* genes are carried in the same direction in both lambda phages, lysogenization through pieces from opposite ends of phage Mu integrates the *lac* genes in opposite directions. Only the lysogen having the *lac* gene integrated in the same direction as the *argA* gene was expected to give the desired fusions. Heat induction of the Mu *cts* prophage yields a deletion at the insertion site of Mu, occasionally fusing the *lac* genes to a nearby promotor. Fusions of the *lac* genes to the *argA* promotor were selected on minimal medium containing lactose under conditions of at least partial derepression of the *argA* gene. This was achieved by adding an arginine precursor, *N*-acetyl-L-ornithine, instead of arginine. This compound is taken up slowly, giving rise to arginine in growth-rate-limiting amounts. Strain 4100A lysogenic for either λp1(209) or λp123(209) yielded Lac$^+$ offspring upon heat induction, but only fusions of strain 4100A lysogenic for λp1(209) became Lac in the presence of arginine. Thus, in λp1(209) the *lac* genes were fused to *argA* with the same reading strand of deoxyribonucleic acid (DNA). The frequency of the Lac$^+$ colonies was 5×10^{-8}, and more than 90% were under the control of *argA*. The enzymatic activities for two fusion strains are listed in Table 2. The repression of β-galactosidase synthesis in both fusion strains is dependent on an active arginine repressor and the presence of arginine. The repression coefficients were 180 for the AZ-1 fusion strain and 350 for the AZ-7 fusion strain, based on the enzyme levels of the corresponding *argR*$^+$ and *argR* derivatives. The AZ-1 fusion strain showed a sevenfold-higher β-galactosidase activity under derepressed conditions than did strain AZ-7. A similar discrepancy has been observed among fusions in the *ara* region (2). For *N*-acetylglutamate synthetase, the enzyme coded by *argA*, the repression coefficient is at least 250 (3).

Isolation of lambda phages carrying *argA-lac* fusions. Induction of lambda phages in *argA-lac* fusion strains resulted in a heterogeneous phage population with a low titer (10^5 to 10^6 plaque-forming units per ml). Fusion strain 4100 AZ-1 yielded about 40% phages carrying the *lac* genes as judged by faint blue plaques formed on X-gal medium. Fusion strain 4100AZ-7 gave only about 10 to 20% blue plaques, which, in contrast to lambda phages from fusion strain AZ-1, were dark blue, indicating a higher β-galactosidase activity during phage multiplication. Either fusion strain AZ-1 produced a less stable β-galactosidase or the rate of synthesis of β-galactosidase was lower during phage multiplication. DNA from phage λAZ-1 (see below) used as template in an in vitro protein-synthesizing system was a poor template in contrast to DNA from λAZ-7 (8). Restriction nuclease analysis of λAZ-1 and λAZ-7 DNAs showed that λAZ-1 carries a large portion of *trp* genes between the *argA* operator and the *lac* genes (T. Eckhardt, unpublished data), which might reduce the efficiency of the *lacZ* transcription. From both fusion strains one blue plaque was isolated. For both phages λAZ-1 and λAZ-7, the intensity of the blue color in the plaques formed during phage multiplica-

TABLE 2. *Enzymatic activities of argA-lac fusions under different conditions*

Strain genotype	Fusion	Growth conditions (minimal medium + growth requirements)	Sp act[a]	
			β-Galactosidase	Ornithine[b] transcarbamylase
4100AZ-1 *argA argR*$^+$	AZ-1	+L-Arginine (100 μg/ml)	15.9	0.028
		+*N*-acetyl-L-ornithine (50 μg/ml)	2,470	16.1
EC113(λAZ-1) *argR argD*[c]	AZ-1	+L-Arginine (100 μg/ml)	2,800	20.5
		−Arginine	2,910	19.1
4100AZ-7 *argA argR*$^+$	AZ-7	+L-Arginine (100 μg/ml)	1.4	0.030
		+*N*-acetyl-L-ornithine (50 μg/ml)	341	16.2
EC113(λAZ-7) *argR*$^+$ *argD*	AZ-7	+L-Arginine (100 μg/ml)	1.2	0.033
		−Arginine	420	19.7
EC113(λAZ-7) *argR argD*	AZ-7	+L-Arginine	492	19.2
		−Arginine	478	18.7

[a] Micromoles of product formed per milligram of protein per minute.

[b] Used to check repressibility of strain by arginine.

[c] Integration of the λAZ-1 or λAZ-7 in the *argA* region; *argD* mutants are leaky and permit physiological derepression.

tion was independent of the presence or absence of arginine, although a repressible strain (MC4100) was used as indicator. It seems either that, during phage multiplication, escape synthesis of β-galactosidase under the control of the arginine repressor takes place or that *N* gene promotor readthrough occurs starting from the p_L promotor of lambda. The latter would be expected, based on the phage structure (Fig. 1d). The heterogeneity of the phage population with respect to their DNAs, as judged by the occurrence of white and blue plaques on X-gal medium after the first induction, is apparently a consequence of the lack of an attachment site or homologous sequences on both sides of the integrated phage, resulting in inefficient excision. The excision seems to yield variable end points as indicated in Fig. 1c. About 5% of the lambda phages from fusion strain AZ-7 formed plaques on a P2 lysogen, showing a Spi (sensitivity to P2 interference) phenotype. In that case, a part of the lambda *red* functions might have been left behind in the chromosome. Multiplication of phage by superinfecting an appropriate host (MC4100) with a single plaque gave a stable phage line. The phages produced only blue plaques on X-gal medium, indicating that the *argA-lac* fusion is carried stably by the lambda phage.

Transcription direction of the *argA* gene. To determine the transcription direction of the *argA* gene on the *E. coli* chromosome, strain EC113, carrying a *lac* deletion, was lysogenized with λAZ-7. The only way for it to become lysogenized stably is through recombination in the *argA* region, using the homology between phage and chromosomal DNA (Fig. 2A). This puts the *argA-lac* fusion on either side of *argA*, depending on the direction of transcription of the *argA* gene (Fig. 2b). To distinguish between the two possible arrangements, the order of *thyA*, *argA*, and *argA-lac* on the chromosome was determined by transduction with phage 363. To do this, an *argA* mutation was introduced by phage 363 transduction. A *lacY* mutation in the *argA-lac* fusion was isolated and used to determine the position of the *argA-lac* fusion relative to *argA* and *thyA*. The mapping data are presented in Table 3. Because the transduction involves a lambda phage in the donor as well as in the recipient, some precautions were taken to insure that all transductants were recovered. One such precaution was to express the donor marker before selection to avoid induction of the lambda phage by starvation (see Materials and Methods). Although the lambda lysogens themselves are very stable, a small fraction among the transductants was always found to have lost the entire lambda phage for unknown reasons. It is possible that this kind of transduction favors cured offspring, although a lambda lysogen donor was used to provide complete chromosomal homology between donor and recipient. Transductants that lost the lambda phage were not scored for mapping. The cotransduction frequency between

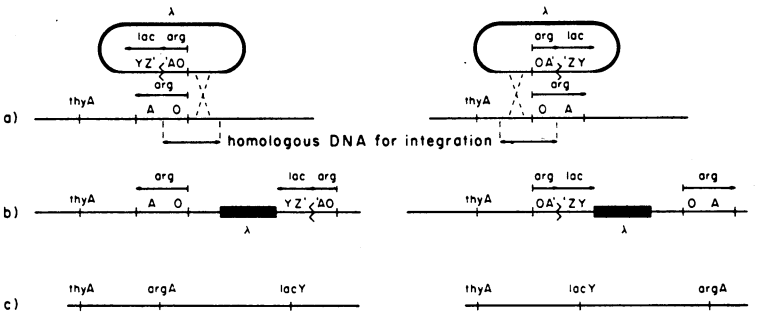
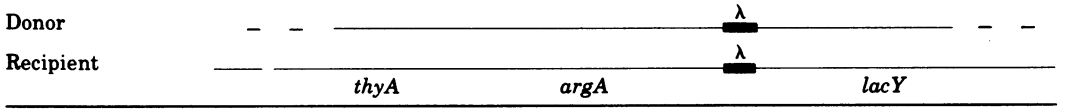


FIG. 2. *Integration of a lambda phage carrying an argA-lac fusion in the argA region, showing the two possibilities depending on the direction of transcription of the argA gene in the E. coli chromosome. Left, Clockwise direction; right, counterclockwise direction. (a) Alignment of lambda carrying an argA-lac fusion with the argA region. (b) Relative order of thyA argA and the argA-lac fusion as result of the direction of transcription of argA (for symbols see legend to Fig. 1). (c) Postulated order of markers used in the three-point mapping.*

TABLE 3. *Order of the argA-lacZY fusion with respect to argA and thyA determined by transduction with phage 363*



Donor marker[a]	Recipient marker[a]	Selected marker (no. isolated)	Cotransduction frequencies of unselected donor markers (%)	
thyA⁺ argA lacY⁺	*thyA argA⁺ lacY*	ThyA⁺ (403)	18 *argA*	0 *lacY⁺*
thyA argA⁺ lacY	*thyA⁺ argA lacY⁺*	ArgA⁺ (299)	19 *thyA*	2 *lacY*
thyA⁺ argA⁺ lacY	*thyA argA lacY⁺*	ThyA⁺ (197)	20 *argA⁺*	0 *lacY*
		ArgA⁺ (297)	24 *thyA⁺*	0.7 *lacY*
thyA⁺ argA⁺ lacY⁺	*thyA argA lacY*	ThyA⁺ (200)	18 *argA⁺*	0 *lacY⁺*
		ArgA⁺ (200)	12 *thyA⁺*	0.5 *lacY⁺*

[a] Donor and recipient strains are the strains EC 90 through EC 95 listed in Table 1.

argA and *thyA* was lower than the expected 40%. The mapping data, however, indicate clearly that *argA* and *thyA* are cotransduced without the *argA-lac* fusion. The fusion is cotransducible at very low frequency with *argA* but not with *thyA*. The low frequency of cotransduction between *argA* and the *argA-lac* fusion was expected since the lambda phage in between is about half the size of phage 363. The gene order is *thyA-argA-lacYZ' 'argA*. From these results the direction of *argA* transcription can be deduced to be clockwise (Fig. 2b, left) on the *E. coli* chromosome.

This finding has been confirmed by a different approach. The direction of the Mu *cts* phage in strain 4100A was found to be (−) by the chromosomal mobilization method of Zeldis et al. (12). According to definition (5), the *c* end of a (−) Mu prophage is clockwise to the *S* end on the *E. coli* chromosome (Fig. 3a). λp1(209), which carried the *c* end of phage Mu, yielded fusion of the *lac* genes to *argA*. Recombination of λp1(209) with strain 4100A can therefore be inferred to give the structure indicated in Fig. 3b. Thus, the *lac* genes are transcribed in a clockwise direction, and, to give functional *argA-lac* fusions, the *argA* gene must be transcribed in the same direction.

DISCUSSION

The isolation of lambda phages carrying *argA-lac* fusions has been helpful in studying regulation of the arginine biosynthetic enzymes. The *argA-lac* DNA provides a suitable assay system for the arginine repressor (8) because of the favorable properties of β-galactosidase in an in vitro enzyme-synthesizing system. Fusion to the *argA* operator provides a very sensitive assay for repressor activity because there is a high repression coefficient for enzyme synthesis with a low level for complete

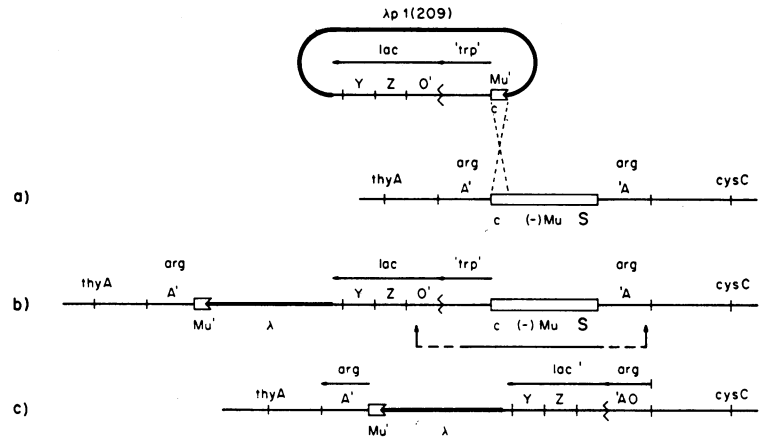


FIG. 3. *Steps in the formation of an arg-lac fusion in strain 4100A (argA::-Mu cts). (a) Lysogenization of phage λp1(209) through the c end of phage Mu. (b) Lambda-Mu lysogen; the lac genes are indicated to be transcribed in a clockwise direction. (c) Final argA-lac fusion (symbols as in Fig. 1).*

repression (3). Furthermore, strains carrying an *arg-lac* fusion give a clear-cut change on different indicator media and allow one to recognize regulatory mutants.

Lambda phages carrying *argA-lac* fusions differ mainly in two aspects: the extent and the nature of the DNA between the *lacZ* gene and the *argA* operator and the amount of chromosomal DNA besides the *argA-lac* fusion picked up during phage excision (Fig. 1c). The restriction enzyme cleavage pattern of lambda phages carrying different *argA-lac* fusions can be compared. Because of the similarity of the phage DNA from different fusions, the *argA-lac* fusion-bearing DNA segment can readily be identified. Since the cleavage sites in *lacZ* for a number of restriction enzymes are known, the *argA* operator-bearing segments can be identified and eventually used for sequencing of the *argA* operator region. Heteroduplex mapping of lambda phages carrying fusions of the *lac* genes to different arginine control regions would allow one to determine the relatedness of different arginine control regions.

Phages carrying gene fusions are useful for determining the direction of transcription of a particular gene on the *E. coli* chromosome. The determination of the direction of transcription of genes that are not part of an operon or gene cluster has been difficult and restricted to special cases. One approach is to isolate operator or promotor mutants and to map them precisely (6). These mutants, however, are difficult to obtain in most cases. Another approach is to map different nonsense mutations and compare the size of their gene products (4, 9). This method involves an elaborate genetic and biochemical approach. The isolation of *lac* fusions to a given operator is a general technique and not restricted to particular cases. It only requires that the regulation of the gene being studied is known in order to verify fusion to its control region. The determination itself involves three-point mapping as a standard genetic technique. Because two of the relevant markers are separated by a lambda phage (Fig. 2b), thereby reducing the cotransduction frequency drastically, the data obtained are clear-cut.

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