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Genetic Characterization of the *araE* Gene in *Salmonella typhimurium* LT2

JAR-HOW LEE, SHEIKHA AL-ZARBAN, AND GARY WILCOX*

Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, California 90024

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Six L-arabinose transport-deficient mutants of *Salmonella typhimurium* LT2 were isolated on the basis of their inability to ferment low concentrations of L-arabinose. The mutations were localized between *serA* and *lys* on the *S. typhimurium* genetic map and assigned to the *araE* locus. An *araE-lac* fusion strain was constructed and used to determine that the direction of *araE* transcription was counterclockwise on the *S. typhimurium* genetic map. β-Galactosidase activity was induced by L-arabinose in the *araE-lac* fusion strain, suggesting that *araE* expression is controlled at the level of transcription.

L-Arabinose can be utilized as a sole source of carbon and energy by *Salmonella typhimurium* (21). The initial step in L-arabinose metabolism is the transport of the carbohydrate into the cell. A number of mechanisms are known which mediate transport of small molecules into bacterial cells (9). We have used a genetic approach to define the components of the L-arabinose transport system in *S. typhimurium*. Six mutants defective in L-arabinose transport were isolated and shown by P22-mediated transduction to be located at 62 units on the genetic map. The locus was designated *araE*. The *lacZ* gene was fused to *araE* by using a procedure which has recently been developed for creating fusions in *S. typhimurium* (18). A method using homology-facilitated chromosome transfer was used to determine that *araE* is transcribed in a counterclockwise direction.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used are listed in Table 1. P22 HT105/1 *int*201 (2) or P22 *int*4 (24) was used in the transduction experiments.

Media and chemicals. TYE medium was prepared as described (18) and was used for the routine cultivation of bacteria. Minimal medium and MacConkey indicator plates were prepared as described (1, 20). Carbohydrates were added to a final concentration of 0.2% (wt/vol) unless otherwise indicated. Other supplements, added as needed, were 40 μg of L-amino acids, 50 μg of ampicillin (Ap) 15 μg of tetracycline (Tc), and 250 μg of streptomycin per ml.

Calbiochem L-arabinose (A grade) was used to prepare minimal L-arabinose medium, and Sigma L-arabinose was used in the preparation of MacConkey indicator plates. MacConkey agar base was purchased from Difco. Lactose and glycerol were from Mallinckrodt. Tetracycline, streptomycin, and glucose were from Sigma. Ampicillin was from the Wyeth Laboratories, Inc.

Mutagenesis, ampicillin enrichment, and screening for Ara⁻ mutants. Isolated clones of strain L001 were mutagenized for 30 min by the addition of 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml as previously described (22). An ampicillin enrichment in minimal L-arabinose medium was performed on the mutagenized culture as described (20). The ampicillin-enriched culture was plated on MacConkey L-arabinose plates. Clones unable to utilize L-arabinose were obtained and further characterized.

Preparation of phage lysate and transductional crosses. Preparation of P22 transducing lysates was performed as described (22). Tranductional crosses mediated by P22 were performed as previously described (18).

Mating experiments. (i) Plate mating was performed by either spotting or spreading the donor and recipient strains on the selective medium (7, 20). (ii) In some experiments broth matings were performed. Overnight cultures of donor and recipient strains were diluted 20-fold into TYE and allowed to grow for 2 h at 37°C. The donor and recipient strains were then mixed at a ratio of 1:10 and incubated without aeration for 1 h at 37°C. The mating was interrupted by vigorous agitation for 1 min. (iii) Complementation tests were performed on minimal plates containing 0.05% L-arabinose as previously described (11). Complementation was indicated by a confluent patch of growth appearing at the intersection after 24 h of incubation at 37°C.

Construction of *araA araE* mutants. The *araE* mutants were transduced to tetracycline resistance by using a P22 lysate cycled on strain TT206, which had Tn*10* inserted in *leu*. A Leu⁻ transductant from each *araE* mutant was purified. The *araE leu*::Tn*10* strains were transduced to Leu⁺ by a P22 lysate cycled on an *araA* mutant, L6004. The Leu⁺ transductants were streaked on a MacConkey plate containing 1% L-arabinose. The *araE araA* mutants showed a negative

TABLE 1. *S. typhimurium LT2 strains*

Strain	Genotype[a]	Reference
L001	*trpD10 cysB12*	(19)
L6004	*trpD10 cysB12 araA4*	This laboratory
L6018	*trpD10 cysB12 araC1*	This laboratory
L6011	*trpD10 cysB12 araE1*	This study
L6021	*trpD10 cysB12 araE2*	This study
L6031	*trpD10 cysB12 araE3*	This study
L6041	*trpD10 cysB12 araE4*	This study
L6051	*trpD10 cysB12 araE5*	This study
L6061	*trpD10 cysB12 araE6*	This study
L6211	*trpD10 cysB12 araA4 araE1*	See text
L6221	*trpD10 cysB12 araA4 araE2*	See text
L6231	*trpD10 cysB12 araA4 araE3*	See text
L6241	*trpD10 cysB12 araA4 araE4*	See text
L6251	*trpD10 cysB12 araA4 araE5*	See text
L6261	*trpD10 cysB12 araA4 araE6*	See text
L7152	*trpD10 cysB12 thyA*	Trimethoprim-resistant derivative of L001
L1119	F$^+$::Mu *d*(Ap^r *lac*)/*trpD10 cysB12 ara-1*	(19)
L0338	F'338 *araE*$^+$ *lys*$^+$/Δ*leu-5111 ara-9 gal-205 fol-107 lys*::Tn*10*	See text
L0418	*trpD10 cysB12 araE*::Mu *d*(Ap^r *lac*) *ara-1*	See text
L0419	F'*ts114 lac*$^+$ *zzf-535*::Tn*10*/*trpD10 cysB12 araE*::Mu *d*(Ap^r *lac*) *ara-1*	See text
L0420	*trpD10 cysB12 araE*::Mu *d*(Ap^r *lac*)	See text
L0421	*trpD10 cysB12 araE*::*lac* Ap^r Δ(Mu *lys mutH*)	See text
L0423	*trpD10 cysB12 araE*::Mu *d*(Ap^r *lac*)*[b]	See text
L0444	F'*3382 araE*$^+$ *lys*$^+$/Δ*leu-5111 ara-9 gal-205 fol-107 rpsL lys*::Tn*10*	See text
L0447	F'*3382 zzf-535*::Tn*10*/*serA790 lys-554* Δ*his-644*	See text
L0443	Δ*leu-5111 ara-9 gal-205 fol-107 rpsL lys*::Tn*10*	See text
L8271	Δ*leu-5111 ara-9 gal-205 fol-107 lys*::Tn*10*	TT215 × TR2330
TT142	*argG*::Tn*10*	J. R. Roth
TT173	*cysC*::Tn*10*	J. R. Roth
TT206	*leu*::Tn*10*	J. R. Roth
TT215	*lys*::Tn*10*	J. R. Roth
SA486	HfrK3 *serA13 rfa-3058*	K. E. Sanderson
SA534	HfrK4 *serA13 rfa-3058*	K. E. Sanderson
JL631	*serA790 lys-554* Δ*his-644*	J. Ingraham
NK1017	F'*ts114 lac*$^+$ *zzf-535*::Tn*10*/*pyrC7 rpsL*	N. Kleckner
311	*lys*	S. Kustu
TR2330	Δ*leu-5111 ara-9 gal-205 fol-107*	J. R. Roth

[a] Genetic nomenclature is as described by Chumley et al. (7), Bachman and Low (3), and Sanderson and Hartman (23).

[b] * indicates that the Mu *d*(Ap^r *lac*) was stabilized.

fermentation response on this medium, but *araE* mutants showed a positive response.

Isolation of an *araE-lac* fusion strain. The isolation of the fusion strain was performed as described (18) with some minor modifications. Strain L1119 containing F$^+$::Mu *d*(Ap^r *lac*) was partially induced at 37°C for 9 h. The culture was then diluted 10-fold, and 0.1 ml was spread on a minimal glycerol (0.1%, final concentration) plus L-arabinose (0.02%, final concentration) plate. Strain L1119 was inhibited on this medium because of the arabinose-sensitive mutation. Only the small colonies that appeared after 48 h of incubation at 30°C were further tested on the following solid media: (i) minimal glycerol (0.1%, final concentration) plus L-arabinose (0.02%, final concentration); (ii) minimal glycerol (0.1%, final concentration) plus L-arabinose (1%, final concentration); (iii) MacConkey lactose; (iv) MacConkey L-arabinose; and (v) MacConkey L-arabinose plus lactose. The results were scored after 24 h of incubation at 30°C.

Stabilization of *lac* structural genes in the *araE-lac* fusion strain. Since the Mu *d*(Ap^r *lac*) phage contains a temperature-sensitive repressor, the phage induced at the nonpermissive temperature will kill most of the host cells. Temperature-resistant survivors might represent strains in which part of the Mu phage genome has been deleted. Alternatively, they may represent a strain in which an X mutation has occurred (6). An overnight culture of the fusion strain, L0420, was diluted 10-fold, and 0.1 ml was spread on a MacConkey lactose (1%, final concentration) plate supplemented with ampicillin. The plate was first incubated at 44°C overnight and then shifted to 37°C for another day.

Curing of the F factor with acridine orange and testing the F⁻ phenotype. The curing of the F factor with acridine orange was performed as described (20). The acridine orange-treated cells were streaked on a TYE plate. Isolated colonies were then checked for the appropriate genetic markers (18).

β-Galactosidase assay. β-Galactosidase activity was determined as described previously (20).

RESULTS

Isolation and characterization of transport-negative mutants. A bacterium which is defective in carbohydrate transport has a cryptic phenotype with respect to the ability to ferment that carbohydrate. We searched for mutants defective in L-arabinose transport by analyzing the fermentation response on indicator medium containing different concentrations of L-arabinose. Twenty-nine Ara⁻ mutants, isolated independently as described in Materials and Methods, were streaked on MacConkey indicator plates containing either 1 or 0.2% L-arabinose. Of the 29 mutants screened, 6 were Ara⁺ on the 1% L-arabinose plate and Ara⁻ on the 0.2% L-arabinose plate. These six strains, perhaps defective in L-arabinose transport, were further studied. The presumed transport-negative mutants were streaked on minimal medium containing different concentrations of L-arabinose. Growth was observed after 48 h of incubation at 37°C only when the L-arabinose concentration was greater than 0.05%. The *araA4* mutation, which is defective in the gene coding for L-arabinose isomerase, was crossed into each of the *araE* mutants to prevent metabolism of L-arabinose. When tested in the standard transport assay (10), all of the presumed transport-negative mutants were unable to accumulate L-arabinose when compared with the parent strain (Table 2). An *araC* mutant, L6018, was also unable to accumulate L-arabinose.

The transport mutants were mapped with the generalized transducing phage P22. All of the transport-negative mutations in *S. typhimurium* were cotransducible with *thyA* and assigned to the *araE* locus. The cotransduction frequencies varied from 18 to 30% depending upon the particular *araE* mutation. Two-factor crosses were used to determine that the order of *araE* with respect to adjacent genes was *serA-araE-lys-thyA* (Table 3). The order of the *araE* mutations was determined by reciprocal three-factor crosses using *lys*::Tn*10* as an outside marker (22). The order was *araE2-araE4-araE3-araE6-*(*araE1*, *araE5*)*-lys*::Tn*10* (data not shown). The order between *araE1* and *araE5* could not be determined since the difference in the experimental data was not significant.

Isolation and shortening of an F′ plasmid containing the *araE* gene. An F′ plasmid containing the *araE* gene was required to perform complementation tests. Selection of an F′ plasmid by using the standard procedure, involving early transfer of a terminal gene from an Hfr strain with the *araE* mutants as recipients, was not successful, probably because of the low concentration of L-arabinose used in the selection

TABLE 2. *L-Arabinose transport activities of ara mutants*[a]

Strain	Mutation(s)	L-Arabinose transport activity[b]	
		Uninduced	Induced
L6004	*araA4*	0.02	0.38
L6018	*araC1*	0.01	0.02
L6211	*araA4 araE1*	0.01	0.03
L6221	*araA4 araE2*	0.01	0.02
L6231	*araA4 araE3*	0.01	0.01
L6241	*araA4 araE4*	0.02	0.05
L6251	*araA4 araE5*	0.02	0.02
L6261	*araA4 araE6*	0.01	0.01

[a] The cells were prepared as described in the text.
[b] L-Arabinose was added as the inducer to a final concentration of 0.4% (wt/vol). A unit of transport activity is the number of micromoles of L-arabinose accumulated per milligram of protein at the steady-state level (15 min).

TABLE 3. *Transductional mapping of araE, serA, lys, and thyA*[a]

Donor strain and mutation	Recipient strain and mutation	Selected marker	No. of transductants with unselected marker:			Total transductants scored	Cotransduction frequency (%)	Map units[b]
			Lys⁻	Ara⁻	Ser⁻			
L6051 (*araE5*)	SA534 (*serA13*)	Ser⁺		0		879	0	
SA534 (*serA13*)	311 (*lys*)	Lys⁺			0	200	0	
L6051 (*araE5*)	311 (*lys*)	Lys⁺		1,153		1,347	86	0.03
L6051 (*araE5*)	L7152 (*thyA*)	Thy⁺		134		480	28	0.35
L311 (*lys*)	L7152 (*thyA*)	Thy⁺	213			552	39	0.26

[a] Transductions were performed as described in the text. The transductants were replica plated to different media to check for unselected marker after 24 h of incubation at 37°C.
[b] Map units were calculated as described by Kemper (16).

medium (0.05%) and the high reversion frequency of the *araE* mutants. An alternate approach was attempted in which the *lys* gene was used instead, since *lys* was 86% cotransducible with *araE* (Table 3). SA486, an Hfr strain which transfers *araE* and *lys* as terminal genes, was mated with L8271, an F⁻ strain carrying Tn*10* inserted in *lys*. The mating was interrupted after 1 h of incubation, and the mixture was spread on minimal glucose medium plus leucine and tetracycline. The frequency of Lys⁺ transconjugants was about 10^{-6} per donor. Fifteen Lys⁺ transconjugants were picked and tested for their ability to transfer the *araE* gene by spot-mating on minimal L-arabinose (0.02%) plates. All of the 15 transconjugants were able to transfer *araE*. Ten of the transconjugants were cross-streaked with several Tn*10* insertion auxotrophs to determine the length of the F′. All 10 of the F′ plasmids were found to carry genes from *tyrA* to *ilvA*, a region consisting of at least 25% of the *S. typhimurium* chromosome.

Although the F′ strains were fertile, it was necessary to reduce the size of the F′ factor for use in further experiments. One of the F′ strains, L0338, contained F′338 which carried *rpsL*, the gene coding for the 30S ribosomal subunit protein S12. The wild-type *rpsL* allele is dominant to the streptomycin-resistant allele (5). The experimental rationale was as follows. When a streptomycin-resistant recipient received the F′ plasmid containing the wild-type *rpsL* gene, it would be sensitve to streptomycin. Only a recipient which received an F′ plasmid not containing the *rpsL* gene would survive. Strain L0338 was mated with L0443 at a 1:1 ratio and spread on minimal glucose medium containing leucine and streptomycin. Four fast-growing Lys⁺, streptomycin-resistant transconjugants were picked from a heavy background after 24 h of incubation at 37°C. No growth was observed when the cells picked from the background were streaked on the same medium. The length of the F′ plasmids in these four strains was checked by cross-streaking with Tn*10* insertion auxotrophs. It was found that all four F′ plasmids carried only that portion of the chromosome between, but not including, *cysC* and *argG*.

One shortened F′ factor, F′3382, was transferred from strain L0444 to a tetracycline-sensitive strain, JL631, by selecting for the transfer of *lys*. Tn*10* was recombined into F′3382 by P22 transduction, and the resulting strain was named L0447. Strain L0447 was mated with the F⁻*araE* mutants by spotting on minimal L-arabinose (0.05%) plates. All the transconjugants tested which received the *araE* gene were also tetracycline resistant. The spontaneous Ara⁻ segregants of the tranconjugants on MacConkey L-arabinose (0.4%) plates plus tetracycline were picked and tested for the transfer of *lys* by cross-streaking with strain TT215. Seventy percent (10 of 14) of the Ara⁻ segregants could transfer *lys* and were presumably *araE* homogenotes. An attempt was made to determine whether the six independently isolated *araE* mutations were in one or more complementation groups. However, the experiment was not successful because we did not observe recombination or complementation, suggesting that the F′ plasmids were unstable.

Construction of *araE-lac* fusion strain. A strain containing an *araE-lac* fusion was isolated from strain L1119 as a colony resistant to 0.02% L-arabinose, as described in Materials and Methods. Two hundred small colonies were picked since they were more likely to have the Mu *d*(Ap^r *lac*) phage integrated into the *araE* gene, resulting in inhibition by L-arabinose. One of the 0.02% L-arabinose-resistant colonies was sensitive to 1% L-arabinose and contained β-galactosidase activity which was inducible by L-arabinose. The F factor in this strain was cured by acridine orange, and the cured strain was named L0418. The spontaneous reversion frequency of L0418 on a minimal L-arabinose (1%) plate was 10^{-6}, the same as the parent, L1119. One spontaneous Ara⁺ colony of L0418 was purified and named L0420. The Mu *d*(Ap^r *lac*) insertion site in strain L0420 was found to be 81% cotransducible with *lys* (Table 4). No Ara⁺ tetracycline- and ampicillin-resistant transductants were observed, suggesting that only one copy of the Mu *d*(Ap^r *lac*) phage was present in the strain. The β-galactosidase activity in strain L0420 was induced 600-fold in the presence of

TABLE 4. *Cotransduction frequency of the araE-lac fusion with lys*[a]

Recipient strain	Ampicillin in medium	Selected marker	No. of Ara⁺ transductants	Total transductants scored	Cotransduction frequency (%)
L0420[b]	–	Tc^r	391	481	81
	+	Tc^r	0	91	
L0423[c]	–	Tc^r	1,593	2,020	79
	+	Tc^r	0	325	

[a] P22 HT105/1 *int*201 cycled on strain TT215 was used in the transduction experiments. Transductions were performed by mixing 10^8 cells and 10^9 phage particles and incubating the mixture at the appropriate temperature for 30 min. The mixture was diluted 50-fold, and 0.1 ml was spread on MacConkey L-arabinose (0.2%) plates containing tetracycline.

[b] The experiment was performed at 30°C.

[c] The experiment was performed at 37°C.

0.4% L-arabinose. The β-galactosidase activity in an *araC araE-lac* fusion strain was not inducible and was approximately the same as the uninduced level in the *araC*$^+$ *araE-lac* fusion strain (data not shown).

The Mu *d*(Ap^r *lac*) phage in strain L0420 was transposable and could interfere with further experiments if it transposed to some expressed promoter. Therefore, the *araE-lac* fusion was stabilized by the isolation of spontaneous temperature-resistant mutants as described above. The temperature-resistant colonies in which Mu *d*(Ap^r *lac*) was still transposable showed a positive fermentation response or had positive sectors on MacConkey lactose plates containing ampicillin. Three negative colonies obtained on MacConkey lactose plates plus ampicillin were isolated. β-Galactosidase activity was inducible by L-arabinose in all three mutants. No spontaneous Lac$^+$ sectors were observed after the strains were streaked on MacConkey lactose plus ampicillin plates and incubated at 37°C for 48 h. Many Lac$^+$ sectors were seen when L0420 was streaked under the same conditions. One of the stabilized *araE-lac* fusion strains, L0421, was unique in that (i) it failed to grow unless the minimal media was supplemented with lysine, and (ii) the frequency of spontaneous streptomycin-resistant mutants was 10^{-6} per cell. Apparently, this strain contained a deletion generated by Mu (14) which extended into *lys* and *mutH* (12, 23). In contrast, the other two stabilized *araE-lac* fusion strains had the same nutrient requirements as the parent, L0420. One of the stabilized fusions, L0423, was cotransducible with *lys* and contained only one copy of the *lac* structural and ampicillin resistance genes of the Mu *d*(Ap^r *lac*) phage, since no Ara$^+$ tetracycline-resistant transductants were observed when the indicator plate was supplemented with ampicillin (Table 4).

Determination of transcription direction of *araE*. The F′*ts114 lac*$^+$ *zzf*::Tn*10* plasmid was transferred into strain L0420 from NK1017 by selecting for the transfer of Tc^r. Since the only homologous region between F′*ts114 lac*$^+$ *zzf*::Tn*10* and the chromosome was the *lac* genes, the F′ should be able to mobilize the bacterial chromosome by integrating into the *araE-lac* fusion region. The direction of chromosome transfer through conjugation was determined by the transfer direction of the *lac* gene on the F′ (19) and the direction of the *lac* genes of the *araE-lac* fusion on the chromosome (Fig. 1). The direction of chromosome transfer mediated by F′*ts114 lac*$^+$ was determined to be counterclockwise, since strain L0419 transferred the *cysC* gene at a much higher frequency than it transferred the *argG* gene. In a mating with strain TT173(*cysC*::Tn*10*), 284 Cys$^+$ recombinants were observed, whereas no Arg$^+$ recombinants were observed in a mating with strain TT142(*araG*::Tn*10*). If we assume that Hfr formation occurred as shown in Fig. 1, then the direction of transcription of the *lac* genes in the *araE-lac* fusion strain was counterclockwise. Since the *lac* structural genes are regulated by the *araE* promoter, the transcription direction of the *araE* gene should be the same as that of the *lac* structural genes. Thus the transcription direction of the *araE* gene should also be counterclockwise (from *serA* toward *lys*). The Mu-generated deletion in strain L0421 that extended into *lys* and *mutH* (Fig. 1) is also consistent with a counterclockwise direction of transcription.

DISCUSSION

In *S. typhimurium*, *araE* is the first gene shown to be involved in the L-arabinose transport system. The location of the *araE* gene is at 62 units on the *S. typhimurium* genetic map between *serA* and *lys*. It is 28% cotransducible with *thyA* (*araE5*, Table 3) by P22-mediated transduction, which is comparable to the 85% cotransduction of the *araE* gene in *Escherichia coli* with *thyA* by P1-mediated transduction (13). The *araE* gene in *S. typhimurium* is positively regulated by the *araC* gene product, because an insertion mutation in *araC* abolished the accumulation of L-arabinose (18). The expression of β-galactosidase in the *araE lacZ* fusion isolated in this study was inducible by L-arabinose and dependent upon *araC*. The positive regulation must take place at the level of transcription since β-galactosidase is inducible by L-arabinose in the *araE-lacZ* fusion strain.

The accumulation of L-[^{14}C]arabinose in an induced wild-type culture was 19-fold higher than in an uninduced culture (Table 2), whereas a 600-fold induction of β-galactosidase was observed in the fusion strain. The difference could reflect any or all of the following possibilities: (i) the sensitivity of the assays, (ii) control mechanisms which are exerted after transcription, and (iii) transport of L-arabinose into or out of the cell by systems other than those controlled by *araC* or coded for by *araE*.

The *araE-lac* fusion strain was used to determine that the transcription direction of the *araE* gene was counterclockwise. Transposons have been used to provide homology between plasmid and chromosomal DNA sequences (7, 15). Particular *lac* gene fusions have proved useful for determining the transcription direction of various genes (8, 17, 25). The disadvantages of previous methods were (i) the requirement of well-

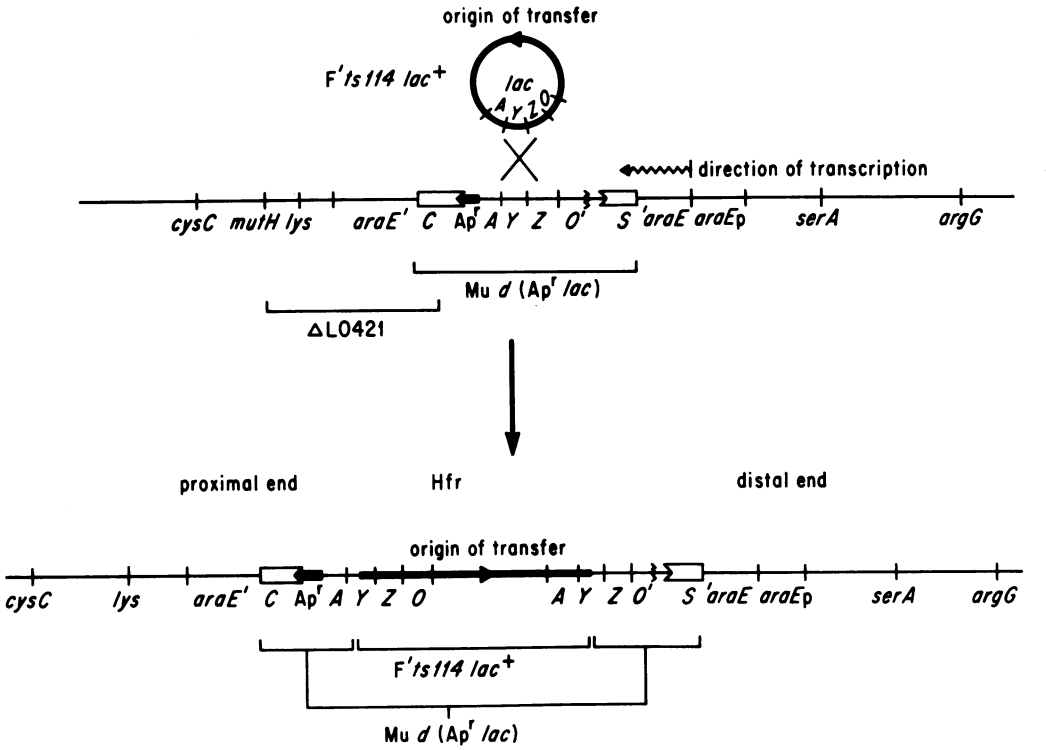

FIG. 1. *Formation of an Hfr plasmid in the araE-lac fusion strain containing F'ts114 lac+. The F' plasmid recombined into the bacterial chromosome through homologous recombination between the two lac structural genes and formed an Hfr strain. The bacterial chromosome was transferred in one direction. ΔL0421 indicates the deletion in strain L0421.*

mapped point mutations in the particular gene, (ii) the requirement of a specialized transducing phage containing the fused gene, and (iii) the restriction to *E. coli* K-12. Using the method described in this paper, the transcription direction of a gene can be easily determined by conjugation. Well-mapped point mutations or specialized transducing phage are not needed. The method can be applied to any bacterial strain that can exchange DNA through F-mediated conjugation and can be applied to genes where well-characterized mutants are not available. A *lac* fusion is the only mutation required in the particular gene for determining the transcription direction.

The spontaneous transpositions fusing the *lac* genes to other expressed promoters are unstable since the Mu *d*(Ap_r *lac*) phage can replicate and transpose in the host cell. Mutants which deleted only part of the Mu genome or X mutants which blocked the replication of Mu phage were isolated by screening for spontaneous temperature-resistant colonies on MacConkey lactose plates containing ampicillin. The temperature-resistant mutants were genetically stable and could be used to study gene expression at all temperatures where the strain was viable.

The procedure used to select F' strains proved to be efficient and in principle could be applied to any region of the chromosome. The use of a *lys*::Tn*10* recipient strain to select F' strains containing the *lys* region had the following advantages: (i) tetracycline present in the medium prevented the growth of spontaneous Lys^+ revertants of the recipient strain which could be generated by precise excision of Tn*10*; (ii) tetracycline could be used as a counterselection for the donor strain; and (iii) recombinants could not survive since medium containing tetracycline without lysine forced the survivors to maintain at least one copy of the wild-type *lys* gene and one copy of *lys*::Tn*10*. The frequency of Tn*10* precise excision and reinsertion at another site on the bacterial chromosome is less than 10^{-9} per cell (4) and therefore is too low to generate a pseudo-recombinant class.

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