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Transposable λ p*lac*Mu Bacteriophages for Creating *lacZ* Operon Fusions and Kanamycin Resistance Insertions in *Escherichia coli*

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We have constructed several derivatives of bacteriophage λ that translocate by using the transposition machinery of phage Mu (λ p*lac*Mu phages). Each phage carries the *c* end of Mu, containing the Mu *c*Its62, *ner* (*c*II), and *A* genes, and the terminal sequences from the Mu *S* end (β end). These sequences contain the Mu attachment sites, and their orientation allows the λ genome to be inserted into other chromosomes, resulting in a λ prophage flanked by the Mu *c* and *S* sequences. These phages provide a means to isolate cells containing fusions of the *lac* operon to other genes in vivo in a single step. In λ p*lac*Mu50, the *lacZ* and *lacY* genes, lacking a promoter, were located adjacent to the Mu *S* sequence. Insertion of λ p*lac*Mu50 into a gene in the proper orientation created an operon fusion in which *lacZ* and *lacY* were expressed from the promoter of the target gene. We also introduced a gene, *kan*, which confers kanamycin resistance, into λ p*lac*Mu50 and λ p*lac*Mu1, an analogous phage for constructing *lacZ* protein fusions (Bremer et al., J. Bacteriol. 158:1084–1093, 1984). The *kan* gene, located between the *c*III and *ssb* genes of λ, permitted cells containing insertions of these phages to be selected independently of their Lac phenotype.

Fusions of genes to the lactose operon of *Escherichia coli* have wide-ranging applications in studies of gene expression and function (1, 23). The use of this methodology has been stimulated by the development of simple procedures for creating *lacZ* fusions in vivo (2–6). We described previously a specialized transducing bacteriophage, λ p*lac*Mu1, that can be used to construct *lacZ* protein fusions in vivo (2). This phage carries terminal sequences from the *c* and *S* (β) ends of Mu (21), including the Mu attachment sites and the Mu *c*Its62, *ner* (*c*II), and *A* (transposase) genes. These sequences allow insertion of the phage genome into bacterial or plasmid chromosomes, resulting in a λ prophage flanked by the Mu *c* and *S* end sequences. A *lacZ* gene, deleted for its promoter and translation start site, is located next to the short Mu *S* end sequence in λ p*lac*Mu1. When this phage inserts into a gene in the correct orientation and reading frame, a protein fusion is created in which *lacZ* is expressed from the promoter and translation start site of the target gene. A hybrid protein is produced by these fusions which consists of the N-terminal sequences encoded by the target gene joined to an enzymatically active C-terminal β-galactosidase polypeptide.

λ p*lac*Mu phages incorporate many of the desirable features of other methods for isolating *lacZ* fusions in vivo into a single system. Strains containing fusions can be isolated in a single step, are genetically stable, and can be conveniently manipulated. Furthermore, they can be directly employed to isolate specialized transducing phages carrying the fusion. In this paper, we describe additional λ p*lac*Mu phages that extend the use of this transposable λ-Mu hybrid phage system. One new phage, λ p*lac*Mu50, can be used to isolate *lacZ* operon fusions. This phage has many of the desirable characteristics of the previously described λ p*lac*Mu phages. However, the *lacZ* gene of λ p*lac*Mu50 contains its own translation start site; hence, only an external promoter is required for *lacZ* expression. In addition, we have introduced a gene that confers kanamycin resistance into λ p*lac*Mu1 and λ p*lac*Mu50. With these phages, insertions can be selected independently of the Lac phenotype.

MATERIALS AND METHODS

Media, chemicals, and enzymes. Luria (L), M63, MacConkey, and tetrazolium media have been described previously (12, 20). Liquid minimal medium containing M63 salts was supplemented with a carbon source at 0.4%. To detect LacZ$^+$ cells and phages, 0.1 ml of a 10-mg/ml solution of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XG) in *N*,*N*-dimethylformamide was added to L agar plates or L soft agar, respectively. Ampicillin, tetracycline, chloramphenicol, and kanamycin were used at 125, 25, 25, and 25 μg/ml, respectively. β-Galactosidase activity was assayed as described by Miller (12).

Strains and phages. The bacteria and phages used are described in Table 1. All strains were *Escherichia coli* K-12 derivatives. λ pSG1 is a derivative of λ p1(209) (4) with a transposon Tn*9* insertion in the *lacY* gene. The Tn*9* insertion allows the selection of λ pSG1 lysogens as chloramphenicol-resistant transductants (20). This phage is also Δ*att*$^\lambda$ and carries the W209 *trpA-lacZ* fusion (13) lacking a functional promoter. It also carries material from the *c* end of phage Mu, which includes the *c* end attachment site and the Mu genes *c*Its62, *ner* (*c*II), and *A* (unpublished data).

Genetic procedures. All basic genetic manipulations followed published procedures (2, 12, 20).

Transposition assays for λ p*lac*Mu phages. Two tests were used to detect transposable λ p*lac*Mu phages. In the single-plaque test, the Δ*lac recA* host strain SE5000 was infected with phage and plated in 5 ml of MacConkey soft agar onto a lactose-MacConkey agar plate. Transposable λ phages

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TABLE 1. Strains and phages

Strain or phage	Description[a]	Reference or origin
E. coli		
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rbsR flbB5301*	4
SE5000	MC4100 *recA56*	Laboratory collection
HS2019	MC4100 Δ*malE444*	18
KLF41	F'141 *malA*⁺/*leuB6 hisG1 recA1 argG6 metB1 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL104 tonA2 tsx-1 supF44*	9
GE1850	MC4100 *uvrD*::Mu dI1(Ap *lac*)	Laboratory collection
GE2085	MC4100 *araBAD*::Mu dII301(Ap *lac*)	2
BRE35	MC4100 *uvrD*::λ p*lac*Mu50	This work
BRE1000	MC4100 *araBAD*::λ p*lac*Mu1	2
BRE1047	MC4100 Φ(*malK-lacZ*)*hyb1002* (λ p*lac*Mul) *malT*::Tn*10*	2
BRE1110	MC4100 Φ(*malT-lacZ*⁺)*1110* (λ p*lac*Mu50)	This work
BRE1113	MC4100 Φ(*malK-lacZ*⁺)*1113* (λ p*lac*Mu50)	This work
BRE1161	MC4100 Φ(*malT-lacZ*⁺)*1110* (λ p*lac*Mu50)	P1 transduction from BRE1110
BRE1162	MC4100 Φ(*malK-lacZ*⁺)*1113* (λ p*lac*Mu50)	P1 transduction from BRE1113
BRE1167	BRE1162 *malT*:Tn*10*	This work
BRE1217	MC4100 (λ p*mal*1161-1)	This work
BRE1219	MC4100 (λ p*mal*1162-1)	This work
Phage		
λ RZ2	*cI*⁺ *att*⁺ Φ(*tyrT-lacZ*⁺) *kan*	R. Zagursky
Mu dI1(Ap *lac*)[b]	See Fig. 1	6
λ pSG1[b]	λ p1(209) *lacY*::Tn*9*	20
λ ap*malB*	*malG*⁺*F*⁺*E*⁺*K*⁺ *lamB' b515 b519 xis*am6 cIts857 *S*am7 *h80*	11
λ ap*malB*Δ1	λ ap*malB* Δ(*malK-lamB*)*1*	16
λ pMu507	cIts857 *S*am7 Mu *A*⁺ *B*⁺	10
λ p*lac*Mul	Mu cIts62 *ner*⁺ *A*⁺ *'ara'* Mu *'S 'lacZ lacY*⁺ *lacA'*	2
λ p*lac*Mu2[c]	λ p*lac*Mul *imm*²¹	This work
λ p*lac*Mu9	λ p*lac*Mul *kan*	This work
λ p*lac*Mu50[b]	See Fig. 1	This work
λ p*lac*Mu51[b]	λ p*lac*Mu50 *imm*²¹	This work
λ p*lac*Mu53[b]	λ p*lac*Mu50 *kan*	This work
λ p*mal*1161-1	Lac⁺-transducing phage from BRE1161	This work
λ p*mal*1162-1	Lac⁺-transducing phage from BRE1162; *malE*⁺	This work

[a] Φ, *lacZ* fusion present; hyb, gene fusion encodes a hybrid protein; *lacZ*⁺ and *'lacZ*, *lacZ* genes with and without translation initiation signals, respectively.

[b] These phages carry a Lac⁻ *trp-lac* fusion that lacks a functional promoter. DNA sequence analysis of the W209 fusion joint between the *trp* and *lac* material (M. Berman, personal communication) revealed that the first 59 codons of *trpA* (24) are joined in frame to codon 3 of *lacZ* (7), resulting in a protein fusion.

[c] This phage is similar to λ p*lac*Mu3 (2) except that it lacks one of the *Eco*RI sites in the λ DNA.

could be recognized after 2 to 3 days of incubation at 37°C by the appearance of Lac⁺ colonies within a single-phage plaque due to the formation of lysogens containing *lacZ* operon fusions. In the spot test, 10-μl portions of phage (~10^5 PFU/ml) were spotted onto 0.2 ml of an overnight culture of strain SE5000 that had been spread on lactose-MacConkey agar. Transposable phages gave numerous Lac⁺ lysogens in the spot after incubation at 37°C for 1 to 2 days. To determine whether λ p*lac*Mu transposition was stimulated by λ pMu507, a phage carrying the Mu *A* and *B* genes (10; M. Howe, personal communication), we added 10 μl of λ pMu507 (10^8 to 10^9 PFU/ml) to the λ p*lac*Mu spot.

When assaying transposition of operon fusion-forming λ p*lac*Mu phages by their ability to form Lac⁺ lysogens, we found it important to use a *recA* mutant host. These phages can also form Lac⁺ lysogens by homologous recombination with the *trp* operon in the *E. coli* chromosome. Use of a *recA* host greatly reduces this recombination; thus, the vast majority of Lac⁺ lysogens selected on a *recA* host are the result of transposition events.

Isolation of λ p*lac*Mu insertions in the *E. coli* chromosome. We mixed 1 ml of a fresh overnight culture of strain MC4100 grown in L medium with approximately 10^8 PFU of λ p*lac*Mu50 and incubated the mixture for 30 min at room temperature. We then added 5 ml of L medium and removed unabsorbed phages by centrifugation. This washing step was repeated three times, and the cells were finally resuspended in 1 ml of L medium. Serial dilutions (10^{-1} to 10^{-4}) were prepared in L medium, and 0.1 ml from each dilution was plated onto lactose-M63 minimal agar to select for Lac⁺ λ p*lac*Mu insertions. Lac⁺ colonies appeared after 2 days of incubation at 37°C. When the λ pMu507 helper phage was used to increase transposition, the same procedure was followed except that the cells were coinfected with λ p*lac*Mu50 (~10^8 PFU) and λ pMu507 (~10^9 PFU). Chromosomal insertions of λ p*lac*Mu phages carrying the *kan* gene (λ p*lac*Mu9 and λ p*lac*Mu53) were isolated by the same method, but the infected cells were plated onto L agar containing kanamycin and XG. No outgrowth for phenotypic expression of kanamycin resistance was required.

When Lac⁺ or Kan^r lysogens of an operon fusion-forming phage were isolated in a *recA*⁺ host, the phage could integrate into the *E. coli* chromosome by homologous recombination at the *trp* operon rather than by transposition. This integration event results in a Trp⁻ phenotype due to disruption of the *trpA* gene. The operon fusions in a *recA*⁺ host described in this paper were isolated on M63-lactose minimal medium, which selects against these Trp⁻ recombinants. We have not determined what fraction of lysogens isolated on rich media was due to *trp* recombination. However, the formation of Lac⁺ operon fusions on MacConkey-lactose medium was stimulated by λ pMu507, suggesting that integration by homologous recombination is not a major event.

Isolation and genetic analysis of Mal⁻ insertions. To isolate λ p*lac*Mu50 insertions in the *malA* or *malB* region, strain MC4100 was infected with λ p*lac*Mu50 alone or in combination with the λ pMu507 helper phage, and Lac⁺ colonies were selected on M63-lactose agar. The Lac⁺ colonies were replica plated onto maltose-tetrazolium agar to identify those that were also Mal⁻ (dark red colonies). Mal⁻ strains were purified twice by streaking on maltose-MacConkey agar, and the Mal⁻ Lac⁺ phenotype was verified with indicator plates and minimal medium. To determine whether a λ prophage was present in these strains, single colonies were cross-streaked against λ *vir* and λ *cI h80*. The Mal⁻ mutations were

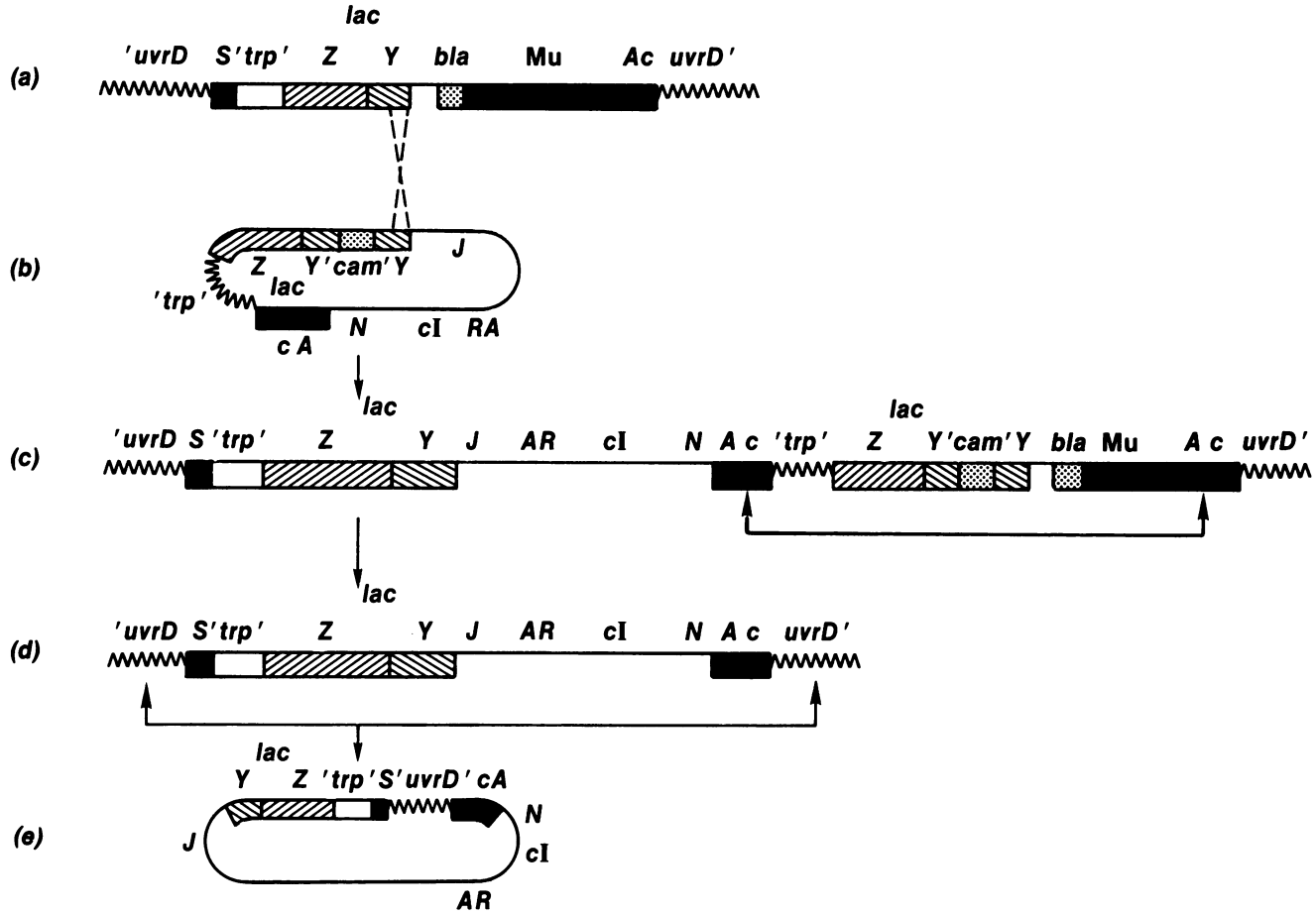

FIG. 1. Construction of λ p*lac*Mu50. Strain GE1850, containing a Lac⁻ insertion of Mu dI1(Ap *lac*) in *uvrD* (a), was lysogenized with λ pSG1 (b). The integration of λ pSG1 shown occurred through *lacY* homology, leading to the formation of a λ pSG1-Mu dI1(Ap *lac*) double lysogen (c). Excision of the Mu dI1(Ap *lac*) prophage by a homologous recombination event involving DNA sequences in the Mu *c* region (c) led to a Lac⁻ λ lysogen, strain BRE35, whose λ prophage was flanked by both Mu attachment sites (d). After UV induction, the λ prophage was excised from the chromosome by an illegitimate recombination event (d and e), resulting in a Lac⁻ λ-Mu-*trp-lac* hybrid phage, λ p*lac*Mu50. Bacterial DNA is shown by a wavy line, Mu sequences by solid black boxes, λ DNA by a thin line, and *lacZ* and *lacY* sequences by striped boxes. The stippled boxes indicate the β-lactamase (*bla*) or the Tn*9*-encoded chloramphenicol transacetylase (*cam*) gene. A prime indicates that a particular gene is not completely present or is interrupted by other DNA sequences. The right-end Mu attachment site is designated *S* and the left-end Mu attachment site is designated *c*.

mapped to the *malA* or *malB* regions by tests described previously (2).

Since multiple insertions of λ p*lac*Mu can occur, it is important that insertions of interest be introduced into a new strain. This prevents possible complications due to the presence of multiple fusions. In the present study, this was accomplished by transducing fusions into strain MC4100 with phage P1 and selecting for Lac⁺ on lactose minimal agar.

RESULTS

Construction of λ p*lac*Mu50. The construction of the translocatable operon fusion-forming λ phage was analogous to that for the protein fusion-forming phage λ p*lac*Mu1 (2). We took advantage of the Mu dI1(Ap *lac*) phage (Fig. 1a) that carries a *lac* operon, deleted for its promoter, adjacent to the Mu *S* end (6). This *lac* operon was derived from the W209 *trp-lac* fusion (13), which is a *trpA-lacZ* protein fusion (M. Berman, personal communication). Translation of the *lacZ* gene in Mu dI1(Ap *lac*) starts at the *trpA* initiation codon and produces a hybrid protein (Table 1, footnote *b*).

The first step was to construct a λ prophage that was flanked by the Mu attachment sites. To do this, we used strain GE1850, which contains a Lac⁻ Mu dI1(Ap *lac*) insertion in *uvrD*. Use of this Lac⁻ strain ensured that the resulting λ p*lac*Mu phage would not confer a Lac⁺ phenotype unless it was transposed and created a fusion. The Mu dI1(Ap *lac*) prophage in strain GE1850 was converted to a λ prophage by the method of Komeda and Iino (Fig. 1) (8). This resulted in the λ lysogen strain BRE35, which contained the desired prophage, i.e, a λ genome with the Mu *S* end (about 200 base pairs [14]) and the *lac* operon on one side and the Mu *c* terminus (about 3,300 base pairs [unpublished data]) on the other (Fig. 1d). These flanking Mu sequences were expected to enable the λ prophage to translocate to new positions when the Mu transposition functions were provided. To test this, we infected strain BRE35 with λ pMu507, a phage carrying the Mu *A* and *B* genes, which encode the necessary transposition functions (21). Transposition of the prophage was monitored by the formation of Lac⁺ colonies, since insertion of the prophage at new locations should generate *lacZ* operon fusions. Numerous Lac⁺ colonies were observed that showed different levels of *lac* operon expression, indicating that fusions to

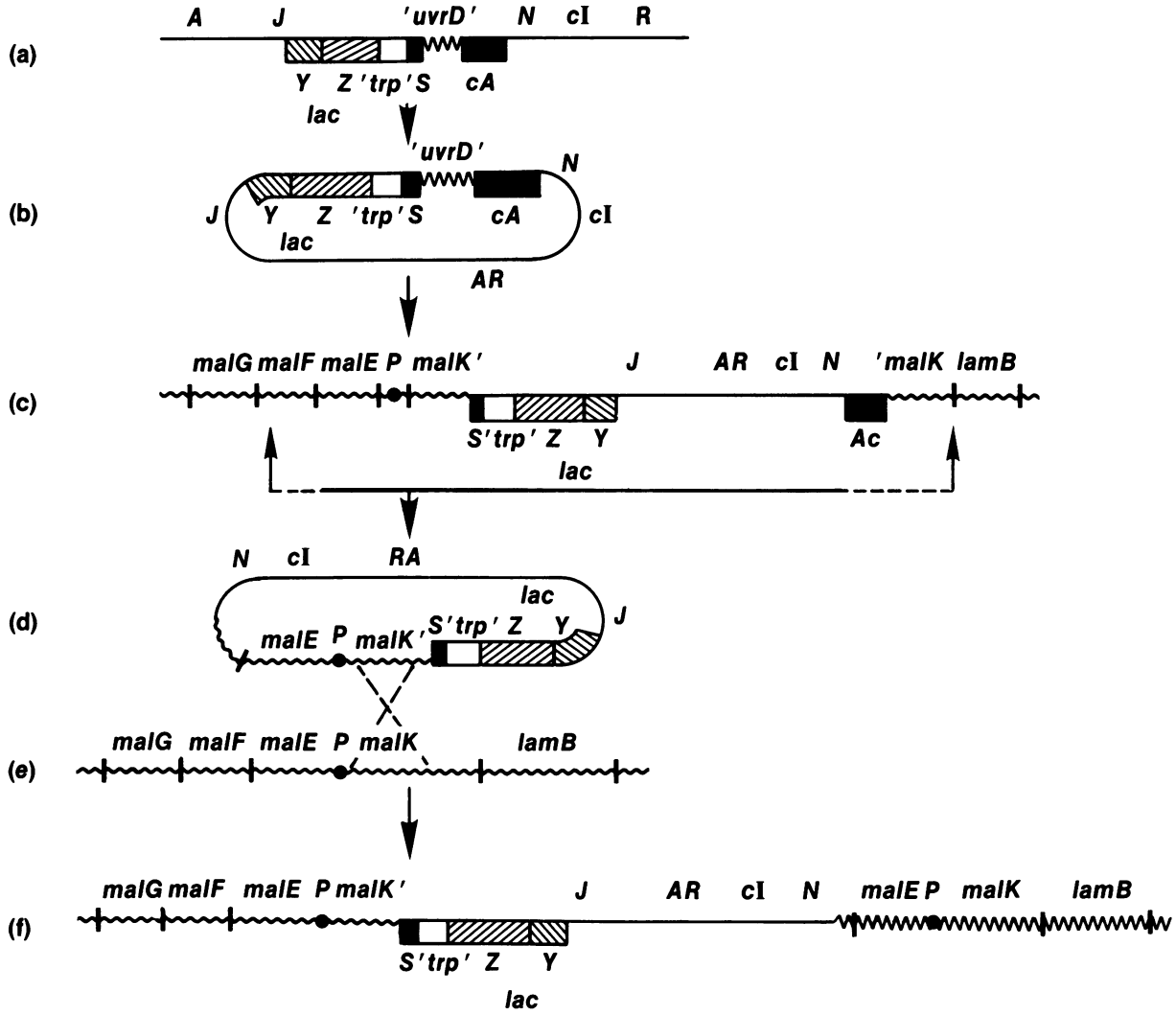

FIG. 2. Insertion of λ placMu50 into *malK* and isolation of a transducing phage. When the mature λ placMu50 chromosome (a) is introduced into the cell, it circularizes (b) and inserts into the *malK* gene (c), resulting in a Lac$^+$ Mal$^-$ *malK-lacZ*$^+$ operon fusion. After UV induction, the prophage is excised at variable end points by an illegitimate recombination event (d), leading to a Lac$^+$ *malK-lacZ*$^+$ specialized transducing phage, which in the phage shown also carries the *malE*$^+$ gene. Infection with this specialized transducing phage leads to Mal$^+$ Lac$^+$ lysogens, formed by integration of the material into the *malB* region by a homologous recombination event (d through f). The black dot represents the two divergent promoters (P) of the *malB* region. All other symbols are described in the legend to Fig. 1.

different promoters had been created. This result suggested that the λ prophage in strain BRE35 can be stimulated to translocate by Mu transposition functions and therefore is flanked by functional Mu attachment sites.

Since the Mu *c* region of λ p1(209), which borders the prophage in strain BRE35, contains a functional Mu *A* gene (2) encoding the Mu transposase (21), phages derived from strain BRE35 containing both the *S* and *c* regions of Mu should transpose by themselves. We previously demonstrated that this is the case in λ placMu1 (2). We took

TABLE 2. Mapping of *mal-lacZ* fusions

Phenotype class[a]	Resistant (R) or sensitive (S) to:		Complementation to Mal$^+$ by:			*lacZ* fusion	No. isolated[b]
	λ cI *h*80	λ *vir*	F'141	λ ap*malB'*	λ ap*malB*Δ1		
1	R	R	+	−	−	*malT*	7 (2)
2	R	S	−	+	+	*malEFG*	26 (14)
3	R	R	−	+	−	*malK*	3 (2)

[a] The class 1 phenotype indicates an *malT* fusion because the strains are complemented to Mal$^+$ by the *malA*$^+$F'141 episome and are resistant to λ *vir* (i.e., *lamB*$^-$). Class 2 fusions are assigned to the *malEFG* operon because they are complemented to Mal$^+$ by either λ ap*malB'* or λ ap*malB*Δ1 and are sensitive to λ *vir*. Class 3 fusions are in *malK* because they are complemented to Mal$^+$ only by λ ap*malB'* and are resistant to λ *vir*. Mal$^+$ recombinants were observed with only two of the class 3 fusions and λ ap*malB*Δ1, indicating that insertions of λ placMu50 in *malK* occurred at different positions.

[b] The number isolated without λ pMu507 helper phage is indicated in parentheses.

advantage of this fact to identify transposable plaque-forming phages derived from the prophage in strain BRE35. The prophage in strain BRE35 was induced with UV radiation, and the resulting lysate was plated on a Δ*lac recA* strain on lactose-MacConkey agar. After several days of incubation, a number of plaques were seen to contain Lac$^+$ lysogens. When the phages in these plaques were purified, they formed white (Lac$^-$) plaques on L agar containing XG. Hence, the Lac$^+$ lysogens were most likely the result of the insertion of a transposable phage into the *E. coli* chromosome to form a *lacZ* operon fusion. When higher-titer stocks of these phages were spotted onto a lawn of a Δ*lac recA* strain on a lactose-MacConkey plate, numerous Lac$^+$ colonies appeared in the phage spots. Moreover, the number of Lac$^+$ colonies was higher and they appeared more rapidly when the phages were spotted together with λ pMu507. This result is expected for a transposable phage, since λ pMu507 carries the Mu *B* gene, which stimulates Mu transposition (15, 21). When the Lac$^+$ colonies were purified from these spots, they were found to be stable λ lysogens and showed various levels of *lac* expression, again indicating that the *lac* structural genes were fused to different promoters. In contrast, phages derived from plaques that did not have Lac$^+$ colonies in their centers were unable to transduce Lac$^+$ regardless of whether the λ pMu507 helper phage was used. We conclude, therefore, that we obtained transposable λ-Mu-*lac* hybrid phages that can create *lacZ* operon fusions in a single step upon integration. In addition, it appears that transposition of these phages can be detected in single plaques. One of these phages, λ p*lac*Mu50, was chosen for further analysis.

Insertion of λ p*lac*Mu50 into the *malA* and *malB* regions. To prove that λ p*lac*Mu50 was a transposable element suitable for isolating *lacZ* operon fusions and to study the properties of fusion strains generated by this phage, we isolated a series of λ p*lac*Mu50 insertions in two defined loci: the *malA* region, encoding the *malP*, *malQ*, and *malT* genes, and the *malB* region, comprising the *malE*, *malF*, *malG*, *malK*, and *lamB* genes (Fig. 2). Transposition of λ p*lac*Mu50 into any of these genes except *lamB* inactivates maltose transport or catabolism and thus results in an easily scorable Mal$^-$ phenotype. Thirty-six Lac$^+$ Mal$^-$ strains were isolated by using λ p*lac*Mu50 alone or in combination with the λ pMu507 helper phage. All strains were found to be λ lysogens, and the Lac$^+$ and Mal$^-$ phenotypes were found to be genetically stable. Analysis of the *mal* mutations in these strains indicated that insertions of λ p*lac*Mu50 into *malT*, *malK*, and the *malEFG* operon had been isolated (Table 2), and no marked preference was obvious when the map positions of the *mal-lacZ*$^+$ fusions were compared against the methods used for their isolation. Therefore, use of the λ pMu507 helper phage did not bias the *lacZ* fusions to a specific gene or region.

TABLE 3. β-Galactosidase assay[a] for λ p*lac*Mu50 insertions in *malA* and *malB*

Strain	β-Galactosidase activity (U)		Induction ratio[b]	Strain description
	Without maltose	With maltose		
MC4100	0	0	0	Δ*lac*
BRE1161	169	205	1.2	Φ(*malT-lacZ*$^+$)*1110*
BRE1217	83	109	1.3	MC4100 (λ p*mal*1161-1)
BRE1162	968	1,137	1.1	Φ(*malK-lacZ*$^+$)*1113*
BRE1167	0	0	0	BRE1162 *malT*::Tn*10*
BRE1219	54	888	16.4	MC4100 (λ p*mal*1162-1)

[a] Strains were grown overnight in glycerol-M63 minimal medium at 37°C. Cells were diluted 1:10 into the same medium with or without 0.4% maltose and grown for 3.5 h at 37°C. The β-galactosidase activity in 0.2-ml samples of the cultures was assayed as described by Miller (12). The difference in basal β-galactosidase levels observed between the original fusion strains and lysogen strains of transducing phages is due to the partially constitutive expression observed in mutants with defects in the maltose transport system.

[b] The induction ratio was calculated as the β-galactosidase units with maltose divided by the β-galactosidase units without maltose.

TABLE 4. Stability of transposable prophages

Strain	Prophage[a]	Frequency[b] of Lac$^+$ derivatives	
		30°C	37°C
GE1850	*uvrD*::Mu dI1(Ap *lac*)	1.2×10^{-6}	1.7×10^{-2}
BRE35	*uvrD*::λ p*lac*Mu50	5.3×10^{-8}	2.3×10^{-7}
BRE1167	Φ(*malK-lacZ*$^+$)*1113* (λ p*lac*Mu50) *malT*::Tn*10*	1.8×10^{-7}	9.0×10^{-7}
GE2085	*araBAD*::Mu dII301(Ap *lac*)	2.2×10^{-6}	9.0×10^{-4}
BRE1000	*araBAD*::λ p*lac*Mu1	$<1 \times 10^{-9}$	2×10^{-8}
BRE1047	Φ(*malK-lacZ*)*hyb1002* (λ p*lac*Mu1) *malT*::Tn*10*	$<1 \times 10^{-9}$	2×10^{-8}

[a] The Mu d(Ap *lac*) and λ p*lac*Mu prophages in strains GE1850 and BRE35, respectively, are located at the same site of insertion in *uvrD*, since strain BRE35 was derived from GE1850 by converting the Mu d(Ap *lac*) phage to a λ phage by the method described by Komeda and Iino (8). The same relationship applies for the prophages in strains GE2085 and BRE1000. Lac$^+$ strains with insertions in *malK* were made Lac$^-$ by introducing the *malT*::Tn*10* mutation.

[b] Strains carrying Lac$^-$ Mu d(Ap *lac*) or λ p*lac*Mu insertions were grown in 0.4% glycerol-containing minimal medium overnight at 30 and 37°C. These cultures were plated on M63-glycerol plates for determining the number of CFU and on M63-lactose plates for selecting Lac$^+$ derivatives. The plates were incubated for 2 days at 30 or 37°C. The values given are the mean values from two independent experiments.

A representative from each class shown in Table 2, isolated by using λ p*lac*Mu50 either alone or in the presence of λ pMu507, was selected for further analysis. None of the strains isolated with λ pMu507 contained this helper phage, and thus we conclude that the helper phage had not integrated into the λ p*lac*Mu507 prophage. When the six *mal-lacZ* strains were transduced to Mal$^+$ with phage P1, four of them gave transductants that were Lac$^-$ and sensitive to λ *vir* and were no longer λ lysogens. This indicates that the prophage and *lacZ*$^+$ fusion were tightly linked to the *mal* mutation in these strains. However, the remaining two strains contained a second prophage, since the Mal$^+$ transductants were Lac$^+$ λ lysogens. Most likely a second insertion of λ p*lac*Mu50 had occurred. Both of these strains were isolated by using λ pMu507. Thus, the stimulation of transposition by the helper phage may increase the frequency of multiple λ p*lac*Mu50 insertions.

Lysates of phage P1 were prepared on all six fusion strains and used to transduce strain MC4100 to Lac$^+$. All Lac$^+$ transductants were found to be Mal$^-$ λ lysogens, and the *mal* mutation in these Lac$^+$ transductants mapped, without exception, in the same position as in the parent strains. Furthermore, when these strains were transduced to Mal$^+$, all of them became Lac$^-$ and were no longer λ lysogens. Since the λ immunity, Mal$^-$, and Lac$^+$ phenotypes of the fusion strains did not segregate from each other, we conclude that λ p*lac*Mu50 transposed into different genes and created *lacZ* fusions. Moreover, since no new insertions were evident in the transductants, it appears that λ p*lac*Mu50 prophages do not transpose at a high level when transduced by phage P1 into a new genetic background.

Regulation of *mal-lacZ*$^+$ operon fusions. To show that the

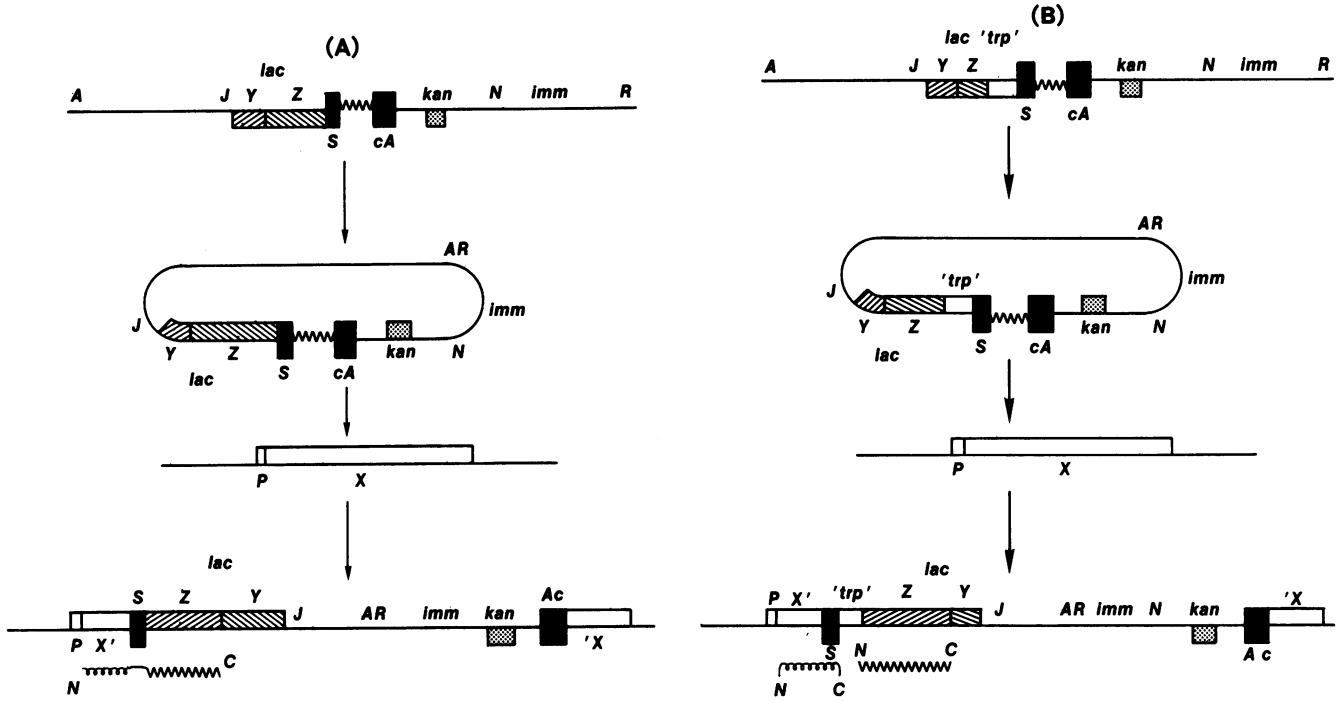

FIG. 3. λ placMu phages carrying a *kan* gene. The integration of (A) λ placMu9 to form a protein fusion and (B) λ placMu53 to form an operon fusion are shown. See the legend to Fig. 1 for explanation of symbols. The proteins produced by each fusion are shown at the bottom.

expression of the *lac* genes in a λ placMu50 insertion reflects the regulatory pattern exhibited by the fused promoter, two tests were used. First, we introduced a mutation that inactivated the positive regulatory element of the maltose region, *malT*, into the strains harboring *lacZ* fusions to *malB*. When a *malT*::Tn*10* mutation was introduced into these strains by P1 transduction, all transductants were found to be Lac$^-$, as shown for one of the *malK-lacZ*$^+$ fusions in Table 3, but they still harbored the λ prophage. Thus, expression of the *lac* genes is regulated by *malT*. Next we determined whether the *mal-lacZ*$^+$ fusions could be induced by maltose. Since this induction requires an intact maltose transport system, we isolated specialized transducing phages carrying the entire *mal-lacZ*$^+$ operon fusion from the different fusion strains and used these phages to construct Lac$^+$ Mal$^+$ merodiploids, as shown in Fig. 2 for a *malK-lacZ*$^+$ fusion. These merodiploids were then shown to have maltose-inducible β-galactosidase activity when the fusion occurred in *malB* but not in *malT* (Table 3). We therefore conclude that *lacZ* operon fusions isolated by the use of λ placMu50 reflect the regulatory pattern of the exogenous promoter. Furthermore, such fusion strains can be employed directly to obtain specialized transducing phages carrying the entire gene fusion.

TABLE 5. Characteristics of λ placMu phage transposition[a]

Infecting phage(s)	Frequency of Kan^r transductants	% Lac$^+$ derivatives
λ placMu9	1.2×10^{-5}	2.7
λ placMu9 and λ pMu507	2.1×10^{-3}	10
λ placMu53	3.4×10^{-5}	50
λ placMu53 and λ pMu507	1.1×10^{-3}	47
λ RZ2	3.1×10^{-4}	100
λ RZ2 and λ pMu507	9.9×10^{-4}	100

[a] Strain MC4100 was grown in L broth at 37°C to an optical density at 600 nm of 0.5. Samples (0.1 ml) of cells were then infected with phage at a multiplicity of infection of 5 to 10. After a 20-min adsorption period at room temperature, 1 ml of L broth was added, and the cells were centrifuged for 2 min at room temperature in a microfuge. The cell pellet was suspended in 1 ml of L broth and recentrifuged. After three washes, the cells were plated on L plates containing kanamycin and XG and incubated at 37°C overnight. A portion of cells was also plated on L plates at 37°C to measure survival and recovery after washing; recovery was always found to be greater than 25%.

Isolation of specialized transducing phages carrying a gene located near the λ placMu50 prophage. Excision of a λ placMu50 prophage after UV induction should occur by an illegitimate recombination event, as is the case for λ placMu1 (2). Therefore, it should be possible to isolate specialized transducing phages carrying intact genes located in the vicinity of the λ placMu50 insertion. To demonstrate this, we tested several Lac$^+$ fusion phages derived from *malK-lacZ*$^+$ fusion strains for the ability to transduce strain HS2019 (*malE*) to Mal$^+$ (Fig. 2c through f). In all cases, *malE*-transducing phages were found. Thus, a λ placMu50 prophage can be used to isolate specialized transducing phages carrying neighboring genes.

Genetic stability of Mu d(Ap *lac*) and λ placMu insertions. We tested the stability of several Lac$^-$ insertions of λ placMu and Mu d(Ap *lac*) phages by measuring the frequency of Lac$^+$ derivatives. We have previously shown (2) that the majority of Lac$^+$ mutants derived from a Lac$^-$ insertion of λ placMu1 in the *ara* locus are the result of insertions of λ placMu1 at new locations. Lac$^-$ λ placMu prophages gave rise to Lac$^+$ mutants at a considerably lower frequency than did Mu d(Ap *lac*) prophages (Table 4). Moreover, the stability of λ placMu prophages was manifested at 37°C, a temperature at which inactivation of the Mu cts62 repressor resulted in a substantial increase in Mu d(Ap *lac*) transposition. This genetic stability of λ placMu insertions should therefore allow the wide range of selection schemes available for the lactose operon (1, 23) to be used to

isolate mutations affecting the expression of the fused target gene.

Derivatives of λ p*lac*Mu phages carrying a gene conferring kanamycin resistance. To introduce a selectable drug resistance gene into the λ p*lac*Mu phages, we used phage λ RZ2, constructed by R. Zagursky. This phage carries a *Sal*I restriction fragment from plasmid pUC71K (22) that contains a gene for aminoglycoside 3′-phosphotransferase (*kan*), originally from Tn*903*. The fragment was inserted in the *Xho*I site of phage λ, between the *c*III and *ssb* genes (17). In addition, phage λ RZ2 carries a *tyrT-lacZ*$^+$ fusion and forms blue plaques on XG indicator media.

Phage λ RZ2 (*imm*$^{\lambda}$ LacZ$^+$) was crossed with λ p*lac*Mu2 or λ p*lac*Mu51 (*imm*^{21} Lac$^-$), and *imm*$^{\lambda}$ LacZ$^-$ recombinant phages were identified by plating the mixture onto a lawn of strain MC4100 (λ *imm*^{21}) on L agar containing XG. Several recombinants from each cross were purified and tested for transposition and for their ability to give kanamycin-resistant (Kan^r) lysogens. One phage from each experiment was chosen for further study: λ p*lac*Mu9 was used to generate Kan^r *lacZ* protein fusions, and λ p*lac*Mu53 was used to generate Kan^r *lacZ* operon fusions (Fig. 3).

Insertions of λ p*lac*Mu9 and λ p*lac*Mu53 could be selected by the Kan^r phenotype of the strains (Table 5). Both Lac$^+$ and Lac$^-$ strains carrying insertions of these phages were recovered. The fraction of Lac$^+$ Kan^r transductants was lower with λ p*lac*Mu9 than with λ p*lac*Mu53, as expected from the additional constraint on the translational reading frame in making a protein rather than an operon fusion. Various levels of *lac* expression among the Lac$^+$ Kan^r transductants were observed for each phage, indicating that fusion to different genes had occurred. These phages, therefore, provide a more general selection for transposition into the bacterial chromosome.

These phages allowed us to quantitate the frequency of obtaining stable λ p*lac*Mu insertions. The λ p*lac*Mu1 phage had been previously found to be Mu *A*$^+$ *B*$^-$ (2). The λ p*lac*Mu phages gave rise to about 1 Kan^r transductant per 10^5 surviving cells, and this was stimulated by one to two orders of magnitude by the λ pMu507 (Mu *A*$^+$ *B*$^+$) helper phage (Table 5). A similar increase in the frequency of transposition of bacteriophage Mu by the *B* gene product has been reported (15, 21). The maximum transposition frequency of these λ p*lac*Mu phages was found to be about 1 transductant per 10^3 surviving cells. We observed that the efficiency of *kan* transduction by λ RZ2, which integrates by the efficient site-specific λ mechanism, was lower than expected and comparable to that of the λ p*lac*Mu phages (Table 5). This suggests that the *kan* gene in some way interferes with the establishment of immunity in lysogeny. The λ p*lac*Mu phages carrying the *kan* gene were found to make clearer plaques than did their parent phages without this insertion, also suggesting a defect in immunity. Despite this, 10^4 to 10^5 transductants can be readily obtained in a single experiment with these phages by using the protocol described.

DISCUSSION

Previously, we described λ p*lac*Mu phages that could be used to isolate *lacZ* protein fusions (2). In the present report, we describe additional phages which extend the range of applications for the λ p*lac*Mu system. λ p*lac*Mu50 is a phage that is suitable for isolating *lacZ* operon fusions. With this phage it should be possible to isolate *lac*$^+$ fusions to untranslated genes (e.g., tRNA or rRNA genes) or to genes which give protein fusions that are deleterious to the cell (e.g., genes encoding exported proteins) (19). Moreover, it is now possible to construct both protein and operon fusions to a gene by using λ p*lac*Mu phages. By comparing the regulation of *lac* expression in strains carrying these two types of fusions, it can be determined whether the regulation of expression of the gene product occurs at the level of transcription or translation. We have also constructed derivatives of these phages carrying a *kan* gene (λ p*lac*Mu9 and λ p*lac*Mu53) to permit insertion strains to be selected by their Kan^r phenotype. This should allow the isolation of strains carrying *lacZ* fusions to genes whose expression is too low to give a selectable Lac$^+$ phenotype, such as repressed genes or genes with an intrinsically low level of expression. In many of these cases, the Lac$^+$ phenotype can still be detected with the indicator XG, which is more sensitive in selecting for the LacZ$^+$ phenotype than lactose. Selecting insertion strains by the presence of the Kan^r phenotype should also be useful for isolating specialized transducing phages carrying a complete gene. In this case, an insertion near the gene of interest is desired, and the Kan^r phenotype provides a general method for selecting such insertions. Taken together, these phages present a convenient and powerful methodology for genetic analysis with *E. coli*.

The λ p*lac*Mu phages that were extensively characterized previously (2) were shown to integrate with little site specificity by the phage Mu transposition system. These insertions were stable and could be isolated in multicopy plasmids. Our results show that the properties of λ p*lac*Mu50 that were tested closely resemble those of these phages. This was expected since all of these phages carry the same Mu *c* material. This segment, derived from λ p1(209), was previously shown to contain a functional Mu *c*-end attachment site and the *c*Its, *ner*, and *A* (transposase) genes, but to lack a functional Mu *B* gene (2). The presence of the *A* gene explains the ability of these phages to transpose by themselves in the absence of any Mu functions provided by helper phages. This intrinsic transposition system does not operate at full efficiency, however, and is stimulated when the Mu *B* function is provided by a helper phage. The absence of *B* and of the neighboring killing function(s) from Mu in these phages undoubtedly accounts for the stable, temperature-resistant phenotype of λ p*lac*Mu lysogens. In contrast, Mu d(Ap *lac*) lysogens which contain these genes are less stable and die at the high temperatures that inactivate the *c*Its repressor, leading to increased expression of these functions. Moreover, the absence of these genes allows insertions of λ p*lac*Mu phages in multicopy plasmids to be recovered (2), which is not the case for Mu d(Ap *lac*) (6). The λ p*lac*Mu50 phage differs from the previously studied λ p*lac*Mu phages in having a slightly longer Mu *S* region. However, this does not appear to affect its behavior as a translocatable element, and we believe that all the λ p*lac*Mu phages will prove to be analogous in their properties.

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