

λ *placMu*: a Transposable Derivative of Bacteriophage Lambda for Creating *lacZ* Protein Fusions in a Single Step

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We isolated a plaque-forming derivative of phage λ , λ *placMu*1, that contains sequences from bacteriophage Mu enabling it to integrate into the *Escherichia coli* chromosome by means of the Mu transposition system. The Mu DNA carried by this phage includes both attachment sites as well as the *cI*, *ner* (*cII*), and *A* genes. λ *placMu*1 also contains the *lacZ* gene, deleted for its transcription and translation initiation signals, and the *lacY* gene of *E. coli*, positioned next to the terminal 117 base pairs from the *S* end of Mu. Because this terminal Mu sequence is an open reading frame fused in frame to *lacZ*, the phage can create *lacZ* protein fusions in a single step when it integrates into a target gene in the proper orientation and reading frame. To demonstrate the use of this phage, we isolated *lacZ* fusions to the *malB* locus. These showed the phenotypes and regulation expected for *malB* fusions and could be used to isolate specialized transducing phages carrying the entire gene fusion as well as an adjacent gene (*malE*). They were found to be genetically stable and rarely ($<10^{-7}$) gave rise to secondary Lac⁺ insertions. We also isolated insertions into high-copy-number plasmids. The physical structure of these phage-plasmid hybrids was that expected from a Mu-dependent insertion event, with the λ *placMu* prophage flanked by the Mu attachment sites. Lac⁺ insertions into a cloned *recA* gene were found at numerous positions and produced hybrid proteins whose sizes were correlated with the position of the fusions in *recA*.

Gene fusions to the *Escherichia coli lacZ* gene (encoding β -galactosidase) provide a powerful tool for molecular biology that has been applied to a broad spectrum of biological problems (2, 47). Two types of fusions have been used: operon fusions, in which an exogenous promoter is fused to a *lacZ* gene lacking its own promoter but containing its own translation start site, and protein fusions, in which a *lacZ* gene lacking both transcription and translation initiation signals is fused in frame to the coding sequence of a target gene. Both types of fusions express *lacZ* from the promoter of the target gene and allow the genetic and biochemical methods developed for the *lac* system to be used to study transcriptional regulation of any gene. In addition, since protein fusions use the exogenous translation initiation signals to express *lacZ*, they can also be used to study translational controls (47). Protein fusions produce a hybrid protein whose N terminus is encoded by the target gene and is fused to an enzymatically active β -galactosidase. These hybrid proteins have additional applications beyond the study of gene expression. For example, hybrid proteins can be used to raise antibodies against the exogenously encoded N terminus, which in turn can be used to identify the product of the wild-type target gene (41). Moreover, hybrid proteins can confer novel phenotypes that can be exploited to dissect the mechanism of a biological process (42) or analyze protein structure-function relationships (30).

Considerable effort has gone into the development of general techniques for isolating *lacZ* gene fusions in vivo (3, 6-8, 17, 27, 28). Most recently, derivatives of the transposable bacteriophage Mu have been constructed that allow either operon fusions [Mu dII(Ap *lac*)] or protein fusions [Mu dII301 (Ap *lac*)] to be isolated in vivo in a single step (8,

9). In these phages a *lacZ* gene, appropriately deleted at its 5' end, and a *lacY*⁺ gene are located a short distance from the *S* (β) end of Mu. When a Mu d(Ap *lac*) phage inserts into a gene in the proper fashion, a fusion is created in which expression from the exogenous signals proceeds through the terminal Mu *S* segment into the *lac* genes. Because the Mu d(Ap *lac*) phages insert into the bacterial chromosome by the Mu transposition mechanism, which shows little site specificity, they can be used to isolate *lacZ* fusions to virtually any gene in *E. coli*.

The Mu d(Ap *lac*) phages have dramatically simplified and generalized the methodology for constructing *lacZ* fusions in vivo. However, the use of these phages does present some problems. First, the ability of Mu d(Ap *lac*) phages to transpose makes insertions of these phages genetically unstable because secondary insertions can occur at a relatively high frequency (14). This complicates certain genetic manipulations such as P1 transduction of a Mu d(Ap *lac*) prophage or selections for rare regulatory mutants based on the Lac phenotype of the fusion. Second, Mu d(Ap *lac*) insertions cannot be directly used to isolate specialized transducing phages carrying the gene fusion. This precludes certain types of genetic analysis and also makes cloning of the fusion difficult. In addition, Mu d(Ap *lac*) lysogens are temperature sensitive for growth because these phages carry a *cI*t_s repressor mutation. Finally, it is not possible to isolate Mu d(Ap *lac*) insertions into genes cloned in high-copy-number plasmids (9). Some of these limitations can be overcome by introducing mutations into the Mu d(Ap *lac*) phages to reduce transposition or temperature-sensitive killing (1) or by converting the Mu d(Ap *lac*) prophage to a λ prophage by homologous recombination (20). However, either these steps do not alleviate all of the above constraints, or they require additional time-consuming manipulations.

To overcome these difficulties, we have isolated a plaque-forming λ phage, λ *placMu*, that can be used to isolate *lacZ*

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TABLE 1. Strains

Strain	Description ^a	Reference or origin
<i>E. coli</i>		
MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>)U169 <i>rpsL150 relA1 deoC1</i> <i>ptsF25 rbsR hbb5301</i>	7
SE5000	MC4100 <i>recA56</i>	Laboratory collection
MBM7014	F ⁻ <i>araC(Am) araD</i> Δ (<i>argF-lac</i>)U169 <i>trp(Am) mal(Am) rpsL relA thi supF</i>	4
GE2085	MC4100 <i>araBAD::Mu dII301(Ap lac)</i>	This work
KLF41	F ['] 141 <i>malA⁺ leuB6 hisG1 recA1 argG6 metB1 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL104 tonA2 tsx-1 supF44</i>	22
HS2019	MC4100 Δ <i>malE444</i>	H. Shuman
pop3194	MC4100 Φ (<i>lamB-lacZ</i>) Hyb52-4 [λ pl(209)]	29
BRE19	MBM7014 <i>srl::Tn10 recA56</i>	This work
BRE1000	MC4100 <i>araBAD::</i> λ <i>placMu1</i>	This work
BRE1002	SE5000 Φ (<i>malK-lacZ</i>) <i>hyb1002</i> (λ <i>placMu1</i>)	This work
BRE1004	SE5000 Φ (<i>malEFG-lacZ</i>) <i>hyb1004</i> (λ <i>placMu1</i>)	This work
BRE1010	SE5000 Φ (<i>malEFG-lacZ</i>) <i>hyb1010</i> (λ <i>placMu1</i>)	This work
BRE1013	SE5000 Φ (<i>malK-lacZ</i>) <i>hyb1013</i> (λ <i>placMu1</i>)	This work
BRE1031	BRE1002(pTM2)	This work
BRE1032	BRE1004(pTM2)	This work
BRE1033	BRE1010(pTM2)	This work
BRE1034	BRE1013(pTM2)	This work
BRE1043	MC4100 Φ (<i>malK-lacZ</i>) <i>hyb1002</i> (λ <i>placMu1</i>)	P1 transduction from strain BRE1031
BRE1044	MC4100 Φ (<i>malEFG-lacZ</i>) <i>hyb1004</i> (λ <i>placMu1</i>)	P1 transduction from strain BRE1032
BRE1045	MC4100 Φ (<i>malEFG-lacZ</i>) <i>hyb1010</i> (λ <i>placMu1</i>)	P1 transduction from strain BRE1033
BRE1046	MC4100 Φ (<i>malK-lacZ</i>) <i>hyb1013</i> (λ <i>placMu1</i>)	P1 transduction from strain BRE1034
BRE1047	BRE1043 <i>malT::Tn10</i>	This work
BRE1048	BRE1044 <i>malT::Tn10</i>	This work
BRE1049	BRE1045 <i>malT::Tn10</i>	This work
BRE1050	BRE1046 <i>malT::Tn10</i>	This work
BRE1057	MC4100 (λ <i>pMal1043</i>)	This work
BRE1059	MC4100 (λ <i>pMal1044</i>)	This work
BRE1060	MC4100 (λ <i>pMal1045</i>)	This work
BRE1068	MC4100 (λ <i>pMal1046</i>)	This work
Phage		
Mu dII301 (<i>Ap lac</i>) ^b	See Fig. 1	8
λ pSG1	λ pl(209) <i>lacY::Tn9</i>	43
λ apmalB	<i>malG⁺F⁺E⁺K⁺lamB'</i> b515 b519 <i>xisam6 cIts857 Sam7 h80</i>	25
λ apmalB Δ 1	λ <i>apmalB</i> Δ (<i>malK-lamB</i>)1	34
λ pMu507	<i>cIts857 Sam7 MuA⁺B⁺</i>	24
λ pMu507.3	<i>imm²¹ Sam7 MuA⁺B⁺</i>	This work
λ <i>placMu1</i>	See Fig. 1	This work
λ <i>placMu3</i>	λ <i>placMu1 imm²¹</i>	This work
λ <i>pMal1043</i>	Lac ⁺ transducing phage from strain BRE1043, also <i>malE⁺</i>	This work
λ <i>pMal1044</i>	Lac ⁺ transducing phage from strain BRE1044	This work

TABLE 1—Continued

Strain	Description ^a	Reference or origin
λ <i>pMal1045</i>	Lac ⁺ transducing phage from strain BRE1045	This work
λ <i>pMal1046</i>	Lac ⁺ transducing phage from strain BRE1046, also <i>malE⁺</i>	This work
Plasmid		
pTM2	ColE1::Tn3 <i>recA⁺</i>	32
pGE142	pBR327 Δ (<i>EcoRI-BamHI</i>)	This work
pGE172	pGE142:: λ <i>placMu3</i>	This work
pGE226	pBR327 Δ (<i>HindIII-AvaI</i>) <i>recA⁺</i>	This work

^a The abbreviation *hyb* indicates that the gene fusion encodes a hybrid protein.

^b DNA sequence analysis of several λ *placMu3* insertions in *recA* revealed that the *MuS* sequence present in this phage is 117 bp long rather than the 116 bp reported for *Mu dII301(Ap lac)* (8). The difference is an extra T between nucleotides 29 and 32 of the published sequence. The resulting sequence in λ *placMu* phages is:

host DNA/TGAAGCGGCGCACGAAAAACGCGAAAGCGTTTCAC.....
10 20 30

protein fusions *in vivo* in a single step. This phage carries the *Mu* attachment sites and inserts into genes nonspecifically by the *Mu* transposition machinery. We have found that fusions isolated with λ *placMu* are stable and temperature resistant, that they can be directly used to isolate specialized transducing phages carrying the fusion as well as genes located near the insertion, and that they can be isolated in genes cloned in high-copy-number plasmids.

MATERIALS AND METHODS

Media, reagents, and enzymes. All liquid and solid media used have been previously described (26, 43). Of a 10 mg/ml solution of 5-bromo-4-chloro-3-indolyl- β -D-galactoside (XG) in dimethylformamide, 0.1 ml was added to agar plates to detect LacZ⁺ colonies. Ampicillin, tetracycline, and chloramphenicol were present in solid media at 125, 25, and 25 μ g/ml, respectively. β -Galactosidase activity was assayed as described by Miller (26). Other enzymes were used according to the specifications of the manufacturer.

Strains. Bacteria, phages, and plasmids are described in Table 1. All bacteria are *E. coli* K-12 derivatives. λ pSG1 (43) is a derivative of λ pl(209) (7) with a Tn9 insertion in the *lacY* gene to facilitate the selection of lysogens. It is Δ *att^l* and carries a *trp-lac* fusion that lacks a functional promoter, as well as material from the *c* end of *Mu*. Plasmid pGE142 is a derivative of pBR327 (45), in which the DNA between the *EcoRI* and *BamHI* sites has been deleted. Plasmid pGE226 is a pBR327 derivative in which a 3-kilobase (kb) *BamHI* fragment carrying the *recA* gene replaces the tetracycline resistance gene (*HindIII-AvaI* fragment) of the vector. This *BamHI* fragment was obtained from plasmid pTM2 (32).

Phage manipulations. λ lysogens were constructed by spotting phage on cells plated on Luria (L) agar plates. After overnight incubation, cells were streaked from the turbid spot onto an L plate seeded with λ *cI h80*. λ immunity was detected by cross-streaking cells against λ *cI h80* and λ *vir*. *Mu* immunity was detected by spotting serial dilutions of *Mu* *cts62* (freshly prepared by temperature induction of a lysogen) on lawns of cells at 30 and 42°C. Plate stocks of λ phages and UV induction of λ lysogens were performed as described (43). Lac⁺ phages were detected by plating in the presence of XG. Stocks of λ pMu507 and λ pMu507.3 were

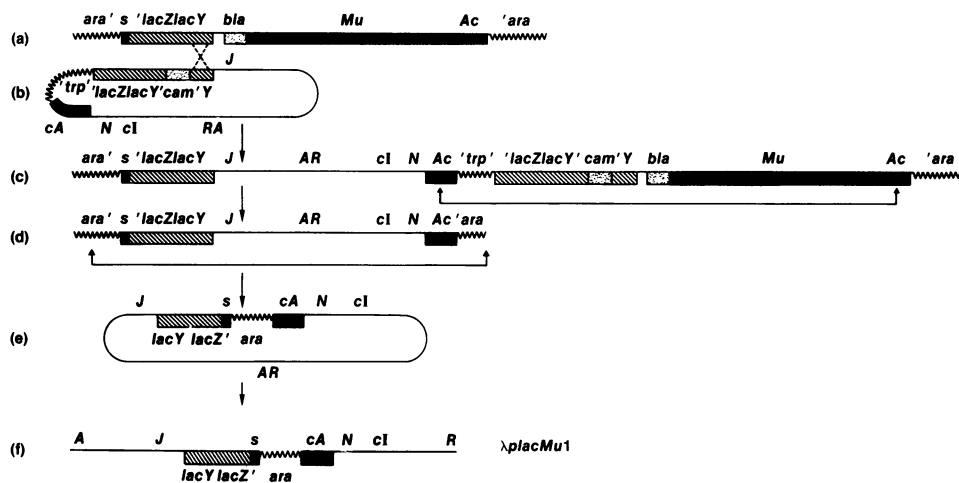


FIG. 1. Construction of λ *placMu1*. Strain GE2085, containing a Lac^- insertion of Mu dII301(Ap *lac*) in the *ara* locus (a), was lysogenized with λ pSG1 (b). The integration of λ pSG1 shown in the figure occurred through *lacY* homology leading to the structure shown in (c). Excision of the Mu dII301(Ap *lac*) prophage by a homologous recombinant event involving DNA sequences in the Mu *c* region (c) led to a Lac^- λ lysogen, strain BRE1000, whose λ prophage is flanked by both Mu attachment sites (d). Upon UV induction, the λ prophage was excised from the chromosome (d and e), resulting in a Lac^- λ -Mu *lac* hybrid phage (f), λ *placMu1*. Bacterial DNA is shown by a wavy line, Mu sequences are represented by black boxes, λ DNA by a thin line, and *lacZ* and *lacY* sequences by a striped box; the shadowed areas symbolize the β -lactamase (*bla*) gene and the Tn9-encoded chloramphenicol transacetylase gene (*cam*). A prime denotes the fact that a particular gene is not completely present or is interrupted by other DNA sequences. The right Mu attachment site is designated *s* (β), and the left Mu attachment site is designated *c*.

prepared on the *supF* strain MBM7014, and stocks of λ *placMu* phages were prepared on strain MC4100.

Construction of GE2085 and BRE1000. Strain MC4100 is sensitive to arabinose because of its *araD* mutation but becomes arabinose resistant when it is also phenotypically $AraB^-$ or $AraA^-$ (6, 11). We used this phenotype to isolate a Mu dII301(Ap *lac*) insertion in the *ara* locus. Strain MC4100 was infected with Mu dII301(Ap *lac*) and plated on arabinose-tetrazolium-ampicillin medium to identify arabinose-resistant insertions. A Lac^- Amp^r Ara^r lysogen was purified, and the Mu dII301(Ap *lac*) prophage was shown to be in the *ara* region by Hfr mapping. This strain was called GE2085. λ pSG1 was then spotted on strain GE2085, and Amp^r Cam^r Mu dII301(Ap *lac*) λ pSG1 lysogens were isolated at 30°C (Fig. 1a to c). Because Mu dII301(Ap *lac*) encodes a temperature-sensitive repressor, these strains die at 42°C. Temperature-resistant Amp^s segregants, which had lost the Mu dII301(Ap *lac*) prophage by homologous recombination, were then isolated at 42°C on L agar (Fig. 1c and d). Both Cam^r and Cam^s Lac^- λ lysogens were obtained, and a Cam^s lysogen (Fig. 1d) was chosen for further study (strain BRE1000).

Isolation of λ *placMu* insertions in the *E. coli* chromosome. Insertions of λ *placMu1* in the *malB* locus were isolated as follows. Serial dilutions of a fresh overnight culture of strain SE5000 were mixed with ca. 10^8 PFU each of λ pMu507 and λ *placMu1*. After adsorption for 20 min at 30°C, the mixture was plated onto lactose minimal agar and incubated for 2 days at 37°C. Those plates containing ca. 1,000 Lac^+ colonies were replica plated onto maltose-tetrazolium agar. After incubation for about 7 h at 37°C, dark red Mal^- colonies could be observed. These were purified on the same medium. The Lac and Mal phenotypes were verified with indicator plates and minimal medium.

There were two drawbacks to this procedure. As reported below, all four *malB* fusion strains that were analyzed in detail were found to have additional λ *placMu* prophages. In addition, we observed that this procedure selected for λ^r

mutants, presumably due to the high concentration of phages on the plates. Two modifications were introduced to overcome these problems. First, 1 ml of cells from an overnight culture was infected at a multiplicity of 0.1 for λ *placMu1* and at 0.1 to 1.0 for λ pMu507. This low multiplicity of infection reduces the frequency of multiple insertions. Second, following adsorption for 30 min at 30°C or room temperature, unadsorbed phages were removed by washing the cells. Two to three washes with 5 ml of L broth were used, and the cells were finally resuspended in 1 ml of L broth. Serial dilutions (10^{-1} to 10^{-6}) in L broth were then plated on lactose minimal medium. This minimized the selection for λ^r mutants.

Several additional observations are pertinent to this procedure. First, the optimal temperature for transposition of λ *placMu* was 37°C. At 30 or 42°C, transposition was greatly reduced. Second, more Lac^+ insertions were recovered when the λ *placMu* phage and helper phage were of the same immunity. The reduced recovery of Lac^+ cells observed with heteroimmune combinations presumably resulted from enhanced killing. We also observed fewer Lac^+ cells in a *supF* strain, most likely because suppression of the *Sam7* mutation of the helper led to increased killing. Finally, the fusions described here were isolated in a *recA* strain. This was done to prevent stable lysogen formation by homologous recombination. However, we have found that this is not a serious problem. The procedure described for the isolation of fusion strains works equally well in *recA^+* recipients.

Isolation of λ *placMu* insertions in plasmids. Two methods have been used to isolate insertions of λ *placMu* into plasmids. The insertions into pGE142 were isolated by preparing a mixed plate stock of λ *placMu3* and λ pMu507.3 on strain MC4100(pGE142) and using the resultant lysate to transduce strain MC4100 to Amp^r . This method allows insertions to be isolated without selecting for a Lac^+ phenotype. Insertions in pGE226 were isolated by infecting plasmid-containing cells with λ *placMu3* and λ pMu507.3 and plating the mix on lactose-MacConkey plates to enrich for

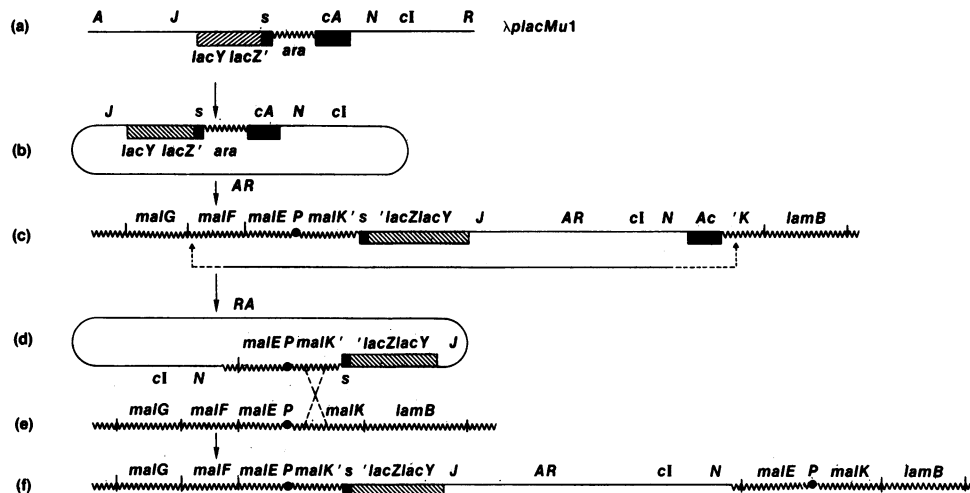


FIG. 2. Insertion of λ *placMu*1 into *malK* and isolation of transducing phages. Upon introduction of the mature λ *placMu*1 chromosome (a) into the cell, it circularizes (b) and inserts into the *malK* gene (c), resulting in a Lac^+ Mal^- *malK-lacZ* gene fusion. Upon UV induction, the prophage is excised by an illegitimate recombination event (d), leading to a Lac^+ *malK-lacZ* specialized transducing phage which also carries the *malE* gene. Infection of this specialized transducing phage leads to Mal^+ Lac^+ lysogens formed by integration into the *malB* region by a homologous recombination event (d to f). This is termed a class I lysogen in the text. Symbols are as in Fig. 1. The black dot represents the divergent promoters (P) of the *malB* region.

Lac^+ cells. After overnight incubation at 37°C, the cells were scraped from the plate into liquid medium, washed several times, then diluted and allowed to grow to exponential phase. A phage lysate was then prepared by induction with UV radiation and used to transduce a *recA* strain to Amp^r . Lac^+ Amp^r transductants were then tested for their degree of UV sensitivity to identify insertions that inactivated the cloned *recA* gene. It should be noted that there is an upper limit to the amount of DNA that λ phage particles may contain. When λ -plasmid hybrid chromosomes exceed this size, the phage particles are unstable, and chromosomes containing deletions are selected. We observed that insertions of λ *placMu*3 into plasmids of 5 to 6 kb or greater often resulted in deletions in the composite chromosomes after transduction.

Genetic analysis of Mal^- insertions. Purified Mal^- λ *placMu*1 insertion strains were first tested for resistance or sensitivity to phage λ by cross-streaking against λ *vir*. To determine whether the Mal^- mutation was in *malA* or *malB*, we performed two tests. Complementation tests were performed with plasmid F'141 carrying a wild-type *malA* region: the Mal^- strains were cross-streaked against strain KLF41, a multiple auxotroph carrying plasmid F'141, on maltose minimal plates; growth in the cross-streaked area indicated complementation for *malA*. Complementation tests were also performed with λ *apmalB*, to test for insertions in *malB*, and with λ *apmalB* Δ 1, to test for insertions in *malK*. These phages were spotted onto a lawn of the Mal^- strain on maltose minimal plates and incubated at 30°C. When complementation occurred, confluent growth of the cells in the spot was seen; when there was no complementation, either no growth or a few isolated Mal^+ recombinants were observed in the spot. To test for the *malE*⁺ gene in specialized transducing phages, we used the same spot method except that strain HS2019, carrying a nonpolar deletion in *malE*, was employed as the bacterial host.

Measurement of the stability of insertions. Strains were grown in glycerol-minimal medium overnight at 30°C, and then titers were determined at 30°C on glycerol-minimal

plates for CFU and on lactose-minimal plates for Lac^+ derivatives.

RESULTS

Transposition of a λ -Mu hybrid prophage. Komeda and Iino (20) described a procedure for converting a Mu d(Ap *lac*) prophage to a λ prophage (Fig. 1a to d). This is accomplished by homologous recombination between the Mu d(Ap *lac*) prophage and the λ transducing phage λ p1(209). The resulting λ prophage is flanked by the termini of Mu: 117 base pairs from the Mu *S* end (8) (see footnote b, Table 1) and ca. 2.8 kb from the Mu *c* end (21). We reasoned that these Mu attachment sites should enable the λ prophage to transpose when the Mu functions necessary for transposition are provided in *trans*. To test this idea, we converted a Lac^- Mu dII301(Ap *lac*) insertion in the *ara* locus to a λ lysogen, strain BRE1000 (Fig. 1), and assayed for transposition by scoring for the formation of Lac^+ colonies, since transposition of the prophage to new locations should generate *lacZ* gene fusions. Mu transposition functions were provided by the λ transducing phage λ pMu507, which carries the Mu *A* and *B* genes (24; Martha Howe, personal communication), known to be sufficient for transposition (46). When λ pMu507 was spotted onto a lawn of strain BRE1000 on lactose-MacConkey agar, numerous Lac^+ colonies were observed. No Lac^+ colonies were visible when wild-type λ was spotted on the same strain or when λ pMu507 was spotted on the nonlysogenic parent strain MC4100. The Lac^+ colonies were found to be stable and showed various levels of β -galactosidase activity, suggesting that a variety of new *lacZ* gene fusions had been generated. To prove that these resulted from translocations, we transduced 10 independent Lac^+ strains to Ara^+ with phage P1. In nine of the strains, Lac^+ Ara^+ transductants were recovered, demonstrating that the Lac^+ fusion was not in the *ara* locus. Thus, the λ prophage in strain BRE1000 can indeed translocate when Mu transposition functions are provided in *trans*.

Isolation of a transposable λ -Mu *lac* hybrid phage. The

TABLE 2. β -Galactosidase assays for λ *placMu1* insertions in *malB*

Strain	β -Galactosidase units ^a		Relevant characteristics
	Without maltose	With maltose	
MC4100	0	2	Δlac
pop3194	157	1,165	$\Phi(lamB-lacZ)$ Hyb52-4
BRE1043	65	64	$\Phi(malK-lacZ)$ Hyb1002
BRE1047	4	3	BRE1043 <i>malT::Tn10</i>
BRE1057	20	178	MC4100 ($\lambda pmal1043$)
BRE1044	257	281	$\Phi(malEFG-lacZ)$ Hyb1004
BRE1048	2	2	BRE1044 <i>malT::Tn10</i>
BRE1059	160	1,131	MC4100 ($\lambda pmal1043$)
BRE1045	202	253	$\Phi(malEFG-lacZ)$ Hyb1010
BRE1049	2	1	BRE1045 <i>malT::Tn10</i>
BRE1060	15	159	MC4100 ($\lambda pmal1045$)
BRE1046	102	112	$\Phi(malK-lacZ)$ Hyb1013
BRE1050	0	0	BRE1046 <i>malT::Tn10</i>
BRE1068	12	126	MC4100 ($\lambda pmal1045$)

^a Cells were exponential-phase cultures grown in glycerol minimal medium with (0.4%) or without maltose. The difference in basal β -galactosidase levels observed between the original fusions and lysogens of transducing phages (e.g., BRE1043 versus BRE1057) is due to a partial constitutivity of expression observed in mutants of the maltose transport system.

above result suggested that a λ phage carrying the Mu attachment sites present in strain BRE1000 should be able to transpose when Mu transposition functions are provided. To isolate such a phage, we induced the prophage in strain BRE1000 by UV irradiation, which produced a lysate of Lac⁻ phages. Since this prophage excises by illegitimate recombination, some of the progeny phages carry both Mu attachment sites (Fig. 1d to f). To determine whether any of these phages could transpose, we tested their ability to form Lac⁺ lysogens by spotting them onto a lawn of strain BRE19 (*recA*) on lactose-MacConkey agar together with λ pMu507. Since transposition of phage Mu is a *recA*-independent process (46), the appearance of Lac⁺ lysogens would indicate transposition of the λ phage into the *E. coli* chromosome. The *recA* host was used to exclude any possible homologous recombination events between the λ phage and the chromosome. Six of sixty phages tested did in fact give rise to Lac⁺ colonies in the phage spots. When single colonies were isolated on lactose indicator medium, stable Lac⁺ lysogens were obtained that showed various levels of *lac* expression, again indicating that the *lac* structural genes

were fused to different genes. We conclude, therefore, that these six phages are λ -Mu *lac* hybrid phages that can transpose when complemented in *trans* by λ pMu507 and can create *lacZ* protein fusions upon integration. One of these phages, λ *placMu1*, was chosen for further study.

Insertion of λ *placMu1* into the *malB* locus. To further characterize the transposition of λ *placMu1*, we isolated insertions in a defined locus, *malB*. This region, along with the *malA* locus, is necessary for maltose catabolism in *E. coli*. The *malB* locus consists of two divergent operons (Fig. 2): one contains the *malE*, *malF*, and *malG* genes, which are necessary for maltose transport, and the other contains *malK*, which is also required for maltose transport, and *lamB*, which encodes an outer-membrane porin that serves as the phage λ receptor and for the uptake of maltose and maltodextrins (34, 35, 44). Inactivation of any of these genes except *lamB* results in a Mal⁻ phenotype.

Lac⁺ Mal⁻ colonies were isolated after coinfection of a *recA* host with λ *placMu1* and λ pMu507 as described above. Twenty of these strains were not complemented to Mal⁺ by the *malA*⁺ episome F'141 and were presumed to have a λ *placMu1* insertion in the *malB* region. These strains all showed a stable Lac⁺ Mal⁻ phenotype and were λ lysogens, but they differed in their levels of β -galactosidase activity. Ten of the strains were resistant to λ *vir*, suggesting that they contained polar insertions in *malK*. The other (λ *vir*^s) strains were candidates for insertions in *malE*, *malF*, and *malG*. None of the strains showed maltose-inducible β -galactosidase activity. This result is to be expected, since a functional maltose transport system is required for induction. Two λ *vir*^r strains (BRE1002 and BRE1013) and two λ *vir*^s strains (BRE1004 and BRE1010) were selected for further study.

To allow further genetic analysis, we transformed the plasmid pTM2, carrying a *recA*⁺ gene, into these strains. When the *recA*⁺ derivatives were transduced to Mal⁺ with the phage P1, only Lac⁻ transductants were obtained. This demonstrated that the Lac⁺ fusions were tightly linked to *malB*. However, all of the Mal⁺ transductants were found to be λ immune, and thus they contained at least one additional λ prophage. Most likely a Lac⁻ insertion of λ *placMu1* had also occurred in these strains. Indeed, prolonged incubation of the Lac⁻ transductants on lactose-MacConkey agar led to the appearance of Lac⁺ papillae resulting from transposition of this prophage. This prophage was not the λ pMu507 (*cl857* Sam7) helper, because the parent *recA*⁺ fusion strains released only phages that formed turbid plaques at 37°C on the *supF* host MBM7014. This result, which was also

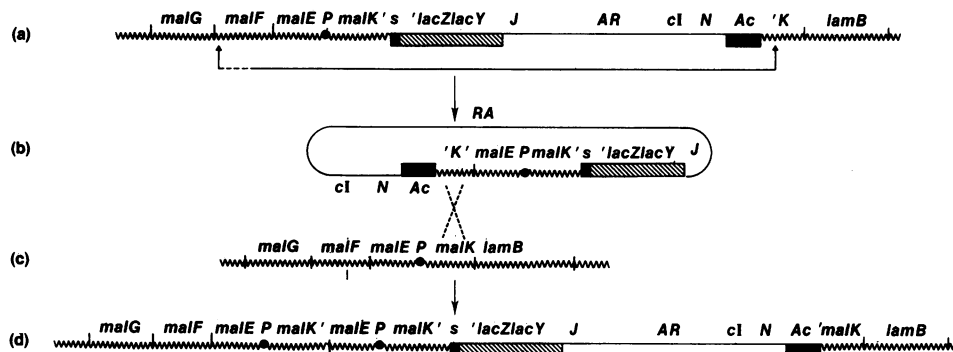


FIG. 3. Formation of Mal⁻ Lac⁺ (class II) lysogens by λ *malK-lacZ* transducing phages. Formation of class II lysogens is described in the text. Symbols are as in Fig. 2.

observed after transposition in *recA*⁺ hosts, also demonstrates that the helper phage does not become inserted into the bacterial chromosome at a high frequency when λ *placMu1* integrates.

To purify the *malB* insertions away from the Lac⁻ prophage, we prepared P1 lysates on the four fusion strains carrying pTM2 and used these to transduce strain MC4100 to Lac⁺. Twenty transductants were purified for each fusion and were found to be Mal⁻ λ lysogens with the same resistance or sensitivity to λ *vir* as the parent strains. Furthermore, when representatives were transduced to Mal⁺, all strains became simultaneously Lac⁻ and were no longer λ lysogens. Thus, these strains (BRE1043 and BRE1046, λ *vir*^r; BRE1044 and BRE1045, λ *vir*^s) now contained only one copy of λ *placMu1*, tightly linked to *malB*. It is also noteworthy that, unlike Mu d(Ap *lac*), λ *placMu1* does not translocate at a high level when introduced to the cell by P1 transduction.

To map the insertions of λ *placMu1* in *malB* more precisely, we used specialized transducing phages carrying parts of *malB*. All four fusion strains could be complemented to Mal⁺ by λ *apmalB*, a phage carrying most of *malB* but lacking the 3' end of *lamB*. The λ *vir*^r strains BRE1043 and BRE1046 were not complemented to Mal⁺ by λ *apmalB* Δ 1, a derivative of λ *apmalB* carrying a deletion starting in *malK* and extending into *lamB*. These results confirm the original conclusion that these strains contain polar λ *placMu1* insertions in *malK*. Mal⁺ recombinants were obtained between strain BRE1046, but not strain BRE1043, and λ *apmalB* Δ 1, indicating that the position of the fusion joint in *malK* is different in these two strains. The λ *vir*^s strains BRE1044 and BRE1045 could be complemented to Mal⁺ by λ *apmalB* Δ 1, consistent with the notion that these strains carry insertions in either *malE*, *malF*, or *malG*. Thus, these experiments prove unambiguously that λ *placMu1* had transposed into several different locations in *malB*.

To prove that these fusions were expressed from the *malB* promoters, we introduced a mutation in the positive regulator of the maltose regulon, *malT*, into each of the four strains. After P1 transduction with a *malT*::Tn10 donor, all tetracycline-resistant transductants were found to be Lac⁻ (Table 2) and still contained the λ prophage. This demonstrates that the expression of β -galactosidase occurs from the *malB* promoters. We therefore conclude that transposition of λ *placMu1* leads to gene fusions expressing *lacZ* from exogenous promoters.

We also tested the λ *placMu1* insertions in *malB* for Mu immunity. The Mu *c* region carried by λ *placMu1* was derived through a series of steps from Mu *cts62* and consequently encodes a temperature-sensitive Mu repressor (7). We found that the λ *placMu1* insertions do confer a temperature-sensitive Mu immunity. This result also shows that no gross rearrangement of the Mu *c* end occurs during integration of λ *placMu1*.

Isolation for specialized transducing phages carrying the gene fusion. The λ *placMu1* prophage carried by the fusion strains cannot excise at high efficiency by either site-specific or homologous recombination. Thus, upon prophage induction, plaque-forming phages should be generated by an illegitimate recombination process that excises the prophage with variable end points. This should produce specialized transducing phages, some of which carry the gene fusion (Fig. 2). To isolate such phages, we induced the four fusion strains BRE1043, BRE1044, BRE1045, and BRE1046 by UV irradiation and plated the resulting phage lysates onto a Δ *lac* strain together with the β -galactosidase indicator XG. In

each case, both Lac⁺ and Lac⁻ phages were obtained. Furthermore, phages both with and without the Mu *cI* gene were observed, as judged by their ability to confer immunity to phage Mu in a lysogen. Thus, excision of λ *placMu1* prophages does indeed occur at variable endpoints.

To demonstrate that the Lac⁺ phages carry the entire *lacZ* gene fusion, including the *malB* promoter, we purified a number of phages and used them to construct Lac⁺ lysogens of strain MC4100. Since these phages do not contain a λ attachment site, they will form stable lysogens by integrating at the *malB* locus by homologous recombination. However, because they carry variable segments of the *malB* region, integration can occur at different positions in *malB*. For example, the Lac⁺ phages isolated from the *malK-lacZ* fusion strain BRE1046 gave rise to two classes of lysogens. Class I lysogens showed a Mal⁺ Lac⁺ λ *vir*^s phenotype. These fusion phages most likely carry the *malB* promoter and have integrated into *malK* in the manner shown in Fig. 2d to f. Class II lysogens were Mal⁻ Lac⁺ λ *vir*^r. This phenotype can be explained if the fusion phage carries not only the 5' end of *malK* but also a segment from *malK* distal to the site of insertion but not extending to the end of the gene (Fig. 3). When integration occurs by recombination in this distal segment, the *malK* gene will be disrupted (Mal⁻), and the *lamB* gene will be separated from its promoter (λ *vir*^r). However, the *malK-lacZ* fusion will be intact (Lac⁺). These results thus demonstrate that one may readily obtain specialized transducing phages carrying *lacZ* gene fusions from λ *placMu1* insertions.

It was not possible to demonstrate maltose-inducibility of β -galactosidase in the parent λ *placMu1* insertion strains (Table 2), because induction of the *malB* promoters by maltose requires a functional maltose transport system. However, in the Mal⁺ Lac⁺ lysogens of Lac⁺ phages isolated from the fusion strains, β -galactosidase was inducible by maltose (Table 2). This result provides further evidence that the Lac⁺ specialized transducing phages carry the original gene fusion. This result also illustrates an important application for phages carrying gene fusions, namely, the construction of merodiploid lysogens, which allow one to study gene expression in a wild-type genetic background.

Isolation of specialized transducing phages carrying genes near the λ *placMu* prophage. As noted above, excision of a λ *placMu1* prophage occurs with variable endpoints. Because of this, it should be possible to isolate specialized transducing phages carrying intact genes located in the vicinity of a λ *placMu1* insertion. To test this, we screened the Lac⁺ phages isolated from the *malK-lacZ* fusion strains BRE1043 and BRE1046 for their ability to transduce the adjacent *malE*⁺ gene. As predicted, *malE*⁺ transduction was observed (data not shown). Thus, λ *placMu1* can be used to isolate phages carrying intact genes as well as fusions.

Insertion of λ *placMu3* into multicopy plasmids. Casadaban and Cohen (9) reported that insertions of Mu dI1(Ap *lac*) into high-copy-number plasmids could not be obtained. To see whether this limitation applied to λ *placMu*, we sought insertions of the phage into pGE142, a derivative of pBR327 in which the material between the *EcoRI* and *BamHI* sites is deleted. To identify insertions of the λ *placMu* phage into this plasmid, we identified specialized transducing phages carrying the entire plasmid by their ability to transduce ampicillin resistance. We first constructed an *imm*²¹ derivative of λ *placMu1*, called λ *placMu3*, to reduce the size of the phage chromosome. This ensured that the hybrid phage-plasmid chromosome would be small enough to be packaged

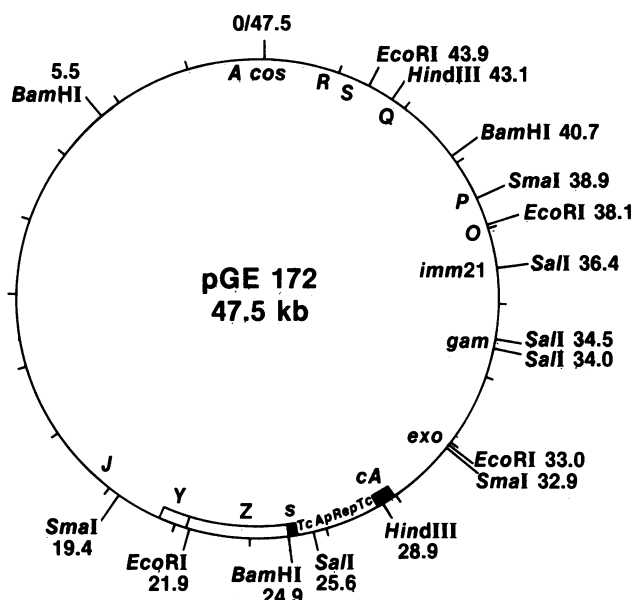


FIG. 4. Physical structure of pGE172. The restriction map of a Lac^- insertion of λ *placMu3* into pGE142, a deletion derivative of pBR327, is shown. The integration of the transposable phage has occurred in the region encoding the tetracycline resistance gene (Tc^r). The positions of the various restriction sites are numbered with respect to their distance from the phage λ *cos* site. The *lacZ* gene, lacking functional expression signals, and the *lacY* gene are represented by the open boxes. The two black boxes mark the position of the Mu attachment sites from the *S* and *c* ends. *Rep* is the plasmid replication region and *Ap* is the β -lactamase gene. To construct the physical map, restriction enzyme digestions of pGE172 were performed with *EcoRI*, *SmaI*, *EcoRI-SmaI*, *BamHI-HindIII*, and *SalI-HindIII*, and the resulting DNA fragment sizes were compared to those predicted from the known λ , *lac*, Mu, and pGE142 sequences. The absence of DNA from the *ara* locus was inferred from the observed restriction fragment sizes. Digests of several other λ *placMu3* insertions, at different positions in pGE142, also confirm this physical structure.

into a phage head. Then a mixed plate lysate of λ *placMu3* and λ pMu507.3 was prepared on strain MC4100 carrying pGE142 and used to transduce a Lac^- recipient to ampicillin resistance. Both Lac^+ and Lac^- transductants were obtained at a frequency of about 10^{-5} transductants per PFU. Thus, insertions of λ *placMu* phages into a multicopy plasmid can be readily obtained. The physical structure of several of these insertions was determined. Plasmid DNA, prepared from the Amp^r transductants by a rapid plasmid isolation procedure (5), was analyzed in detail by restriction enzyme digestion. This revealed that λ *placMu3* had inserted in numerous positions in pGE142. Furthermore, in all cases, the phage was found to have inserted in a unique permutation, flanked by the Mu attachment sites, and the *ara* DNA sequences present in λ *placMu3* (Fig. 1e) were absent. Thus, the physical structure expected for λ *placMu3* insertions was found. A restriction map of a Lac^- insertion (pGE172) of λ *placMu3* into pGE142 is shown in Fig. 4.

We further examined the site-specificity of λ *placMu3* transposition into plasmids by isolating insertions in a defined gene. Twenty-five *lacZ* fusions to the *recA* gene in pGE226 were isolated with λ *placMu3* as described above, and their structure was analyzed by restriction enzyme digestions. Insertions of λ *placMu3* occurred at numerous

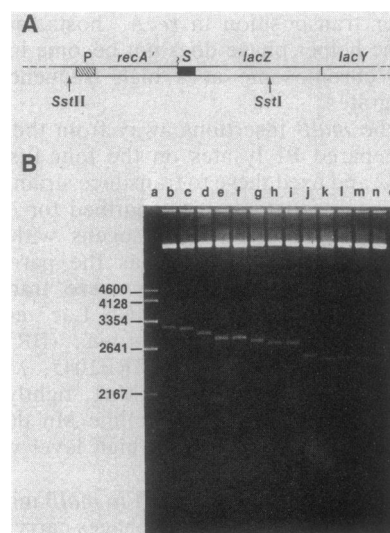


FIG. 5. Restriction enzyme digests of plasmids carrying *recA-lacZ* gene fusions. Plasmid DNA, isolated by a rapid procedure (5), was simultaneously digested with the *SstII* and *SstI* restriction enzymes and subsequently analyzed by agarose gel electrophoresis. (A) An *SstII* site occurs 112 nucleotides before the *recA* promoter (P) (36), and an *SstI* site is present at nucleotide 1947 of *lacZ* (18). (B) Double digestion with these enzymes produces a DNA fragment that can vary in length from ca. 2,200 to 3,300 nucleotides, depending on the position of the λ *placMu3* insertion in *recA*. All other fragments produced by this digest are very large. Lanes a and o are DNA markers. The *recA-lacZ* hybrids used were: b, 125; c, 134; d, 104; e, 102; f, 135; g, 136; h, 123; i, 101; j, 120; k, 132; l, 137; m, 143; n, 138.

sites throughout the *recA* gene (Fig. 5). We therefore conclude that λ *placMu* can insert at many sites in a target gene.

Synthesis of hybrid proteins from Lac^+ gene fusions. Lac^+ gene fusions created by insertions of λ *placMu* phages should contain a variable 5' segment from the target gene fused in frame with the 117-base-pair open reading frame from the Mu *S* end that is in turn fused in frame with *lacZ* at codon 8. As a result, hybrid proteins of variable length should be produced. To demonstrate this, we analyzed extracts from several of the *recA-lacZ* fusion strains described above on sodium dodecyl sulfate-polyacrylamide gels. Each fusion strain produced a hybrid protein (Fig. 6). Furthermore, the size of the hybrid protein increased with the distance of the insertion from the start of the gene. These results prove that λ *placMu* insertions make protein fusions.

Stability of λ *placMu* prophages. As described above, translocation of the prophage in strain BRE1000 is greatly stimulated when Mu transposition functions are provided by a helper phage. In fact, in the absence of a helper, the frequency at which this Lac^- strain gives rise to Lac^+ derivatives is ca. 10^{-8} /CFU. Quite a different result is observed with a Mu dII301(*Ap lac*) prophage. The parent of strain BRE1000, strain GE2085, which contains a Lac^- Mu dII301(*Ap lac*) insertion at the same location in the *ara* locus, gives rise to Lac^+ derivatives at a frequency of ca. 10^{-3} /CFU. Since the λ prophage in strain BRE1000 has the same structure as a λ *placMu1* insertion (Fig. 1d), the Lac^- phenotype of λ *placMu1* insertions should also be very stable. To test this, we used the *malT::Tn10* derivatives of the two *malK-lacZ* fusions described above (strains BRE1047 and BRE1050). These Lac^- strains gave rise to Lac^+ derivatives at frequencies from 2×10^{-7} to 7×10^{-8} /

CFU, comparable to that observed with strain BRE1000. Thus, we conclude that λ *placMu* prophages rarely give rise to new fusions.

Transposition of λ *placMu*1 in the absence of helper phages. The rare Lac⁺ derivatives of strain BRE1000 just described could be due to rearrangements at the original site of insertion or transpositions of the prophage to new locations. To distinguish these possibilities, we transduced nine independent Lac⁺ derivatives of strain BRE1000 to Ara⁺ with phage P1. In all cases, Ara⁺ Lac⁺ transductants could be obtained. This shows that the Lac⁺ fusions were not located in the *ara* locus and, thus, transposition of the prophages must have occurred. We then tested λ *placMu*1 and found that, upon infection, this phage could also transpose in the absence of the λ pMu507 helper phage. Stable Lac⁺ lysogens could be obtained after infecting a Δ *lac* recipient with λ *placMu*1, regardless of whether a *recA*⁺ or *recA* host was used. Approximately 0.1% of the cells infected by λ *placMu*1 became Lac⁺. However, transposition appeared to be reduced in frequency or slower than when the λ pMu507 helper was present. The Lac⁺ fusions isolated in the absence of helper phage showed various levels of β -galactosidase activity on indicator plates, demonstrating that the phage had inserted at different positions. We therefore conclude that λ *placMu* phages contain the functions from phage Mu that are necessary for transposition.

Transposition of λ *placMu* in the absence of the λ pMu507 helper could also be observed in isolated plaques. When λ *placMu*1 was plated on a *recA* host on lactose-MacConkey agar, numerous Lac⁺ papillae were observed in the plaques after a few days incubation at 37°C. These Lac⁺ papillae presumably result from insertion of λ *placMu*1 into the chromosome. We have never observed a λ *placMu*1 plaque that did not show this papillated morphology (several thousand plaques were screened). We conclude that, despite the presence of a functional Mu transposition system, λ *placMu*1 is quite stable and does not generate transposition-defective variants at a high frequency.

DISCUSSION

λ *placMu* provides a simple and generally applicable tool for isolating and manipulating *lacZ* protein fusions. λ *placMu* fusions are formed in a single step when the phage inserts in a gene in the proper orientation and reading frame. These fusions produce hybrid proteins whose expression reflects the regulation of the target gene, demonstrating that *lacZ* expression depends upon exogenous transcription and translation initiation signals. Insertions can be readily isolated in many different genes or at many sites within a gene. Moreover, λ *placMu* can be used for directed transposition into genes cloned into plasmid vectors. Thus, λ *placMu* allows the full range of protein fusion methodology to be applied to any gene in *E. coli*. Since λ *placMu* prophages are stable, they can be subjected to a variety of genetic manipulations (e.g., P1 transduction) and selections without complications arising from secondary transposition events. In addition, transducing phages carrying the fusions can be isolated directly by induction of the λ *placMu* lysogen. Such transducing phages have numerous uses; for example, they facilitate cloning the fusion or, as demonstrated here for the maltose regulon, studying gene regulation. Many of these methods are also possible with the widely used Mu d(Ap *lac*) phages. However, the use of λ *placMu* eliminates the need for the additional time-consuming genetic manipulations that must be performed with Mu d(Ap *lac*) insertions.

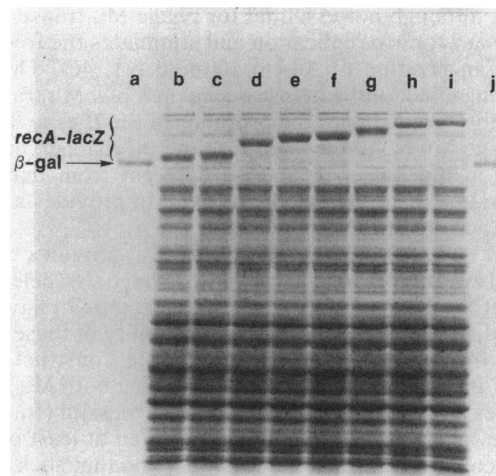


FIG. 6. Hybrid proteins specified by *recA-lacZ* fusions. Extracts from several of the fusions analyzed in Fig. 5 were prepared and run on sodium dodecyl sulfate-polyacrylamide gels as described previously (10). Lanes a and j are wild-type β -galactosidase (12). The insertions used were: b, 138; c, 120; d, 101; e, 136; f, 135; g, 104; h, 134; i, 125.

In addition to its use for isolating gene fusions, λ *placMu* provides a general method for isolating specialized transducing phages. As shown in this paper for the *malE* gene, the ability of λ *placMu* to insert near a gene of interest provides a means to isolate transducing phages carrying the gene. Previously, the ability of the site-specific integration system of phage λ to utilize secondary attachment sites has provided a way to isolate specialized λ transducing phages (39, 40). However, this abnormal λ integration is not random, and thus the isolation of phages carrying certain genes has not been possible (37). With the nonspecific integration of the λ *placMu* phages, this technique should now be applicable to any gene. Furthermore, it should now be possible to routinely construct libraries of λ -*E. coli* hybrids by *in vivo* methods with the same generality that *in vitro* cloning methods offer.

The presence of the Mu attachment sites in λ *placMu* endows this phage with the transposition properties expected of bacteriophage Mu. Thus, insertions can be isolated in either *recA*⁺ or *recA* hosts at many different loci and show a unique DNA permutation, with the Mu attachment sites flanking the prophage. Furthermore, since λ *placMu* can translocate by itself, it must contain a functional Mu A gene. This gene, which encodes the Mu transposase, is necessary and sufficient for transposition of phage Mu (31, 46). The size of the Mu *c* region present in λ p1(209), and therefore in λ *placMu*, has been previously determined by electron microscopy to be about 2.8 kb (21). Based upon available DNA sequence data from the Mu *c* region (33) and the molecular weight of the Mu A gene product (13, 23), it can be calculated that about 3.3 kb of DNA is necessary to encode both the *c*-end attachment site and a complete Mu A gene. Whether the size of the Mu *c* DNA present in λ p1(209) has been underestimated in the studies cited above or whether the transposable phage encodes a truncated but functional Mu A gene is not known. It appears, however, that the Mu *c* region in λ *placMu* is too small to encode a functional Mu B gene. This conclusion is supported by the observation that transposition of λ *placMu* is stimulated by the λ pMu507 transducing phage, which contains functional Mu A and B genes (24; Howe, personal communication). The Mu B gene

product, although not essential for phage Mu transposition, is necessary for Mu replication and stimulates the frequency of Mu transposition by 10- to 100-fold (31, 46). Thus, the stimulating effect of the helper phage on λ *plac*Mu transposition is most likely due to the presence of the *B* gene. Studies with phage Mu have indicated that the mechanism of transposition in the presence of *B* is different from that in its absence (15, 16, 19, 31, 38, 46). λ *plac*Mu provides a simple assay (Lac^+) to study this question.

The $A^+ B^-$ phenotype of λ *plac*Mu provides several practical advantages for the use of this phage. No deleterious effects of the Mu sequences on λ *plac*Mu growth have been observed, nor is there any marked instability of these hybrid phages. In contrast, previous attempts to construct analogous $A^+ B^+$ λ -Mu hybrid phages containing both Mu attachment sites by *in vitro* methods were unsuccessful (16, 38). In this case, only derivatives that had deleted at least one Mu attachment site were recovered, indicating that such $A^+ B^+$ phages cannot be maintained and are unstable. Furthermore, transposition of a λ *plac*Mu prophage occurred less frequently than was the case for a Mu dII301(*Ap lac*) prophage. Inasmuch as the Mu dII301(*Ap lac*) phage is $A^+ B^+$, its increased transposition is probably due to the presence of a functional *B* gene. It is noteworthy, however, that transposition of an infecting λ *plac*Mu phage is more efficient than that of a prophage. This enhanced transposition may reflect differences in the Mu *A* gene dosage, DNA replication, chromosome structure, or the λ gene products present in the cell. In any case, this effect is of tremendous practical importance because it allows insertions to be readily isolated that are then very stable.

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