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Conditionally Transposition-Defective Derivative of Mu d1(Amp Lac)

KELLY T. HUGHES* AND JOHN R. ROTH

Biology Department, University of Utah, Salt Lake City, Utah 84112

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A Mu d1 derivative is described which is useful for genetic manipulation of Mu-*lac* fusion insertions. A double mutant of the specialized transducing phage Mu d1(Amp Lac *c*62ts) was isolated which is conditionally defective in transposition ability. The Mu d1 derivative, designated Mu d1-8(Tpn[Am] Amp Lac *c*62ts), carries mutations which virtually eliminate transposition in strains lacking an amber suppressor. In such strains, the Mu d1-8 prophage behaves like a standard transposon. It can be moved from one strain of *Salmonella typhimurium* to another by the general transducing phage P22 with almost 100% inheritance of the donor insertion mutation. When introduced into a recipient carrying *supD*, *supE*, or *supF*, 89 to 94% of the Amp^r transductants were transpositions of the donor Mu d1-8, from the transduced fragment into new sites. The stability of Mu d1-8 in a wild-type, suppressor-free background was sufficient to permit use of the fusion to select constitutive mutations without prior isolation of deletions to stabilize the fusion. Fusion strains could be grown at elevated temperature without induction of the Mu d prophage. The transposition defect of Mu d1-8 was corrected by a plasmid carrying the Mu *A* and *B* genes.

The specialized transducing phage Mu d1(Amp^r Lac *c*62ts), originally constructed by Casadaban and Cohen (10), has become one of the most effective tools for studying regulation of transcription in bacteria. The simple isolation of *lac* operon fusions to any operon of interest allows an easy means of surveying the regulation of transcription from a particular promoter. The fusions also permit identification of classes of genes with a particular regulatory response (19, 33, 34). Variation in the levels of β-galactosidase activity is directly related to variation in the levels of transcription from the promoter to which the *lac* operon is fused. This is especially useful in studying regulation of functions for which no direct simple assay is possible (20). In addition, methods for selecting *lac* regulatory mutants can be used to isolate regulatory mutants for any gene to which fusion can be achieved (3).

Recently another defective derivative of Mu, Mu d2(Amp^r Lac *c*62ts) (4, 9), has been constructed which is similar to Mu d1 except that insertions of Mu d2 can generate a gene fusion whose product is a hybrid protein derived in part from the target gene and in part from the *lacZ* gene present on Mu d2 (Fig. 1). These fusion proteins have β-galactosidase activity. Fusions of this type have been especially useful in studying protein localization (29).

The ease of forming Mu d1 and Mu d2 insertions (hereafter collectively termed Mu d) is due to: (i) the high frequency with which Mu d transposes, (ii) the randomness of insertion into the chromosome, and (iii) the ability to directly select for transpositions via selection for ampicillin resistance encoded in the Mu d genome (10). Once inserted, the Mu d genome is reasonably stable to further transposition at low temperature due to the synthesis of a thermosensitive repressor protein, a product of the Mu *c* gene (10).

Unfortunately, even at noninducing temperatures (30°C), the Mu d insertion undergoes transposition-rearrangement events at a frequency of 10^{-3} to 10^{-4} (22). This frequency is too high to permit selection for regulatory mutants. Selection for elevated *lacZ* levels usually results in mutants in which Mu d has transposed and formed a new fusion, placing *lacZ* under the control of a more highly expressed promoter.

Another problem with Mu d insertion mutants is that they must be maintained at low temperature, and the original insertion mutation cannot simply be introduced into new strains due to zygotic induction of transposition functions (21). At elevated temperatures, Mu d can kill the host by at least one of two known mechanisms, expression of the Mu *kil* gene or the production of phage replication functions through the loss of *c*ts repressor function (7, 14, 15, 32). Both the temperature restriction and the difficulty of moving fusions into new genetic backgrounds present problems for genetic manipulation of Mu d fusion mutations. To avoid these problems, it has usually been necessary to stabilize each fusion of interest. Stabilization of Mu d insertions has been accomplished by selection for deletions which remove the Mu d genes encoding the transposition functions (8, 20, 21) or by construction of insertion mutants in the Mu *B* gene (2, 7). In the latter case, Mu d is stable to killing at elevated temperatures; however, it is still capable of transposition by zygotic induction due to a fully functional Mu *A* gene (2). In any case, each Mu d insertion to be studied must be stabilized individually and screened to be sure that stability has been achieved. When large numbers of Mu d insertions are isolated, this task is very time consuming.

One alternative method is to stabilize the fusion strains by introducing a plasmid that expresses the wild-type Mu *c* gene (35). Although this allows the strains to grow at elevated temperatures, the disadvantages are that one would have to do all the work in these plasmid-carrying strains, and it has not been demonstrated that this plasmid prevents the high background of replicative transposition which occurs with Mu d1 lysogens. A second alternative is to use a λ Mu-*lac* hybrid for isolating *lacZ* gene fusions (6; E. Bremer, G. Weinstock, and T. J. Silhavy, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, H9, p. 107). This hybrid still carries the Mu *A* gene which allows transposition under zygotic induction conditions; however, the phage lacks the Mu *B* and *kil* genes and thus makes stable fusions. In any case, *Salmonella* species are not hosts for λ and the use of this phage is restricted to *Escherichia coli*.

* Corresponding author.

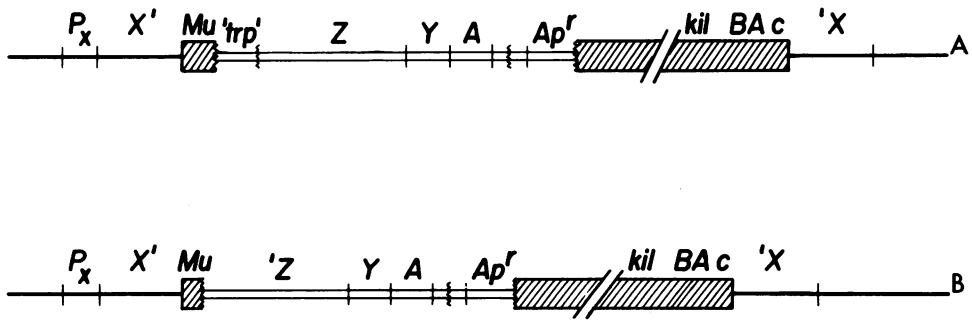

FIG. 1. Structure of fusion phages Mu dl and Mu d2. Mu DNA is presented as a boxed region with slash lines, non-Mu DNA inserted into Mu is presented as a double line, and the host bacterial chromosome is presented as a single line. These phages were constructed by Casadaban and Cohen (10) and by Casadaban and Chou (9), respectively. (A) Operon fusion phage Mu dl inserted into a transcribed region (gene *X*) in the proper orientation results in expression of the Mu dl *lac* operon by transcription initiated from the host promoter, P_X. The *trp* sequences include a translation initiation region. Operon fusions (Lac^+) made with this phage produce a functional *lacZ* protein. (B) Protein or gene fusion phage Mu d2 inserted in an expressed host gene (gene *X*) in the proper orientation and reading frame leads to the production of a novel fusion protein. This protein includes the N-terminal portion of the host protein encoded in gene *X* and a joint region resulting from translation of 116 bases of Mu DNA attached to a β-galactosidase sequence lacking only the first seven base pairs. This figure also illustrates the position of the Mu *c*, *A*, *B*, and *kil* genes.

We describe here a conditionally stable derivative of Mu dl. Under nonpermissive conditions, the element is not subject to either heat killing or zygotic induction and can be easily moved from strain to strain. Under permissive conditions, the new Mu d can transpose, permitting isolation of new *lac* fusions. The mutations causing this conditional stability have been added to both Mu dl (the operon fusion phage) and to Mu d2 (the protein fusion phage).

MATERIALS AND METHODS

Bacterial strains. All strains used in this study and their sources are listed in Table 1. All *Salmonella typhimurium* strains were derived from LT2. *E. coli* PP1073 was constructed and kindly provided to us by P. van de Putte.

Genetic nomenclature. The basic genetic nomenclature used is that described by Demerec et al. (13). *S. typhimurium* TT9749 is an insertion mutant in the *hisD* gene. This has been given a *hsiD* and not a *hisD* allele number. This is an extension of the *his* allele nomenclature. All *his* alleles up to *his-9999* have been used. All subsequent mutants isolated in the *his* operon are given *hsi* allele numbers starting at *hsi-1*.

Media. The E medium of Vogel and Bonner (31) supplemented with 0.2% glucose was used as minimal medium. Difco nutrient broth (8 g/liter; Difco Laboratories) with NaCl added (5 g/liter) was used as rich medium. Difco agar was added at a final concentration of 1.5% for solid medium. Auxotrophic requirements were included in media at final concentrations described by Davis et al. (12). Ampicillin was added to a final concentration of 30 μg/ml in nutrient broth and to 15 μg/ml in E medium. Media containing ampicillin were always prepared fresh before use. 6-Aminonicotinamide and azetidine carboxylate were obtained from Sigma Chemical Co. and used at final concentrations of 50 and 25 μg/ml, respectively. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal; Sigma) dissolved in *N*,*N*-dimethyl formamide (20 mg/ml; Fisher Scientific Co.) was added to media at a final concentration of 25 μg/ml.

Transductional methods. The high-frequency, generalized transducing bacteriophage P22 mutant *HT105/1 int-201* was used for all transductional crosses. This phage was derived by G. Roberts (unpublished results) from the P22 *HT105/1* phage of Schmieger (28). Selective plates were spread directly with 2×10^8 cells and 10^8 to 10^9 phage. Transductants were purified, and phage-free clones were isolated by streaking nonselectively onto green indicator plates (11). Phage-free clones were then checked for phage sensitivity by cross-streaking with P22 H5 (a clear plaque mutant) phage. P1-*vir* phage lysates were prepared and transductions were performed by the method of Berman et al. (5).

Mutagenesis. Hydroxylamine mutagenesis of P22 transducing phage and diethyl sulfate mutagenesis of cell cultures was done as described by Davis et al. (12).

Plasmid mobilization. The plasmid pLP103-6-3, which expresses the Mu *A* and *B* gene functions, was constructed and kindly sent to us by P. van de Putte. The *E. coli* bacteriophage P1 was grown in an *E. coli* strain that harbored this plasmid and which was used to transduce the plasmid into a restriction-deficient, modification-proficient *galE S. typhimurium* strain by selection for ampicillin resistance. *Salmonella* sp. *galE* mutants are sensitive to infection by P1 when grown in media lacking galactose (24). P22 was then grown on this *Salmonella* strain and used to transduce the plasmid into other *Salmonella* strains. The ability of small plasmids to be introduced into strains by generalized transduction has been demonstrated by R. Menzel and R. Johnson (unpublished data).

Mu d mobilization. In *S. typhimurium*, the different Mu d phages described here were moved from strain to strain by P22 transduction (16) (B. Morrison, unpublished data). Mu d lysogens were selected for by selection for Mu d-encoded ampicillin resistance. In *E. coli*, Mu d was transduced by using a Mu helper phage as described by Berman et al. (5) or by P1 transduction of a Mu d lysogen.

RESULTS

Use of Mu dl as a transposon. In selecting transposition-defective mutants of Mu dl, we used the defective Mu dl(Amp Lac *c*62ts) prophage as a transposon (16, 26). No complete Mu helper phage was provided. The Mu dl phage was transduced from strain to strain in *S. typhimurium* by using the general *Salmonella* transducing phage P22 (16) (B. Morrison, unpublished data). The Amp^r gene of Mu dl was used as a selective marker. When donor Mu dl prophage was transduced by P22 into a new recipient, essentially all of the Amp^r transductants were transpositions of Mu dl from the transduced fragment to a new site in the recipient

chromosome. Thus, P22 is capable of packaging and transducing the Mu d1 prophage; Mu helper phage is not required for transposition of Mu d1 in the new recipient.

We used Mu d1 insertions in either of the chromosomal genes *pncA* and *putP*, which causes cells to be resistant to the analogs 6-aminonicotinamide and azetidine carboxylate, respectively. When P22 grown on a donor strain carrying a Mu d1 insertion in one of these genes transduced wild-type LT2 cells to Amp^r, greater than 99.9% of the Amp^r transductants remained analog sensitive as a result of Mu d1 transposition to new sites throughout the chromosome. Thus, transductants resulting from transposition did not inherit the analog-resistant phenotype of the donor insertion mutation.

Isolation of a transposition-defective derivative of Mu d1. P22 lysates were prepared on strains which carry an insertion mutation phenotypically resistant to both ampicillin (due to the presence of Mu d) and to a metabolite analog (6-aminonicotinamide or azetidine carboxylate; (due to the insertion mutation). The phage stocks were mutagenized in vitro with hydroxylamine (17) and used to transduce wild-type LT2 to Amp^r. The Amp^r transductants were then screened for those that had also acquired the donor insertion phenotype (analog resistance). Those that inherited the analog resistance phenotype resulted from either Mu d1 transposition into the same gene or inheritance of the donor insertion mutation by homologous recombination. Mutant Mu d phages which are defective in transposition can only be transduced by homologous recombination; thus, they were expected to contribute to the Amp^r transductants which inherited the donor analog resistance phenotype.

Putative transposition-defective Mu d1 derivatives (Amp^r, analog resistance) were then screened for temperature-sensitive growth. Relief of repression at elevated temperatures results in the death of the host by prophage induction or expression of the *kil* gene. A transposition-deficient Mu d might show temperature resistance, provided a single mutation could affect these properties at elevated temperatures. Mutant Mu d1 prophages which proved deficient in transposition and resistant to high temperature were then tested for suppressibility by amber suppressors.

Three different parental Mu d1 insertion mutants were used in the isolation of amber-suppressible, stable derivatives of Mu d1. Each insertion conferred analog resistance: TT7227 (*pncA207*::Mu d1) and TT7232 (*pncA212*::Mu d1) are resistant to the nicotinamide analog 6-aminonicotinamide (18), whereas strain TT7963 (*putP1017*::Mu d1) is resistant to the proline analog azetidine carboxylate (25). P22 phage stocks were grown on each of these drug-resistant strains and mutagenized with hydroxylamine by the procedure of Hong and Ames (17). These mutagenized phage stocks were used as donors into LT2, with selection for Amp^r transductants. Approximately 1,000 Amp^r transductants from each of the three crosses were screened for analog resistance. Of the 3,000 Amp^r transductants screened, 10 were found to be analog resistant. These 10 were screened for temperature sensitivity by patching onto nutrient broth agar at 42°C. Six were found to be viable at 42°C, suggesting a defect in transposition. Of the six, one was from TT7227, three were from TT7232, and two were from TT7963. These six were then screened for the ability to transpose in a *sup*$^+$ and a suppressor-carrying (*supE*) strain. This was tested by P22-mediated transduction of the mutant Mu d1 as a donor into recipient strains with and without a *supE* suppressor (DB7155 and DB7136, respectively). In each case, Amp^r transductants were selected, and the percentage of transductants showing analog resistance was scored. One of the original six candidates (*pncA212*::Mu d1-6; from TT7232) was found to transpose at a high frequency in the *supE* recipient; less than 0.1% of the Amp^r transductants inherited the donor analog resistance. In the recipient with no suppressor, 57% of the Amp^r recombinants which inherited Mu d1-6 showed the 6-aminonicotinamide resistance phenotype (Table 2). Thus, Mu d1-6 is defective but not completely deficient in transposition ability.

TABLE 1. *S. typhimurium* and *E. coli* strains used

Strain	Genotype	Source[a]
S. typhimurium		
LT2		Lab collection
DB7136	*hisC527 leuA414*	D. Botstein
DB7155	*hisC527 leuA414 supE*	D. Botstein
TT7227	*pncA207*::Mu d1	
TT7232	*pncA212*::Mu d1	
TT7575	*pncA212*::Mu d1-6	
TT7610	*zeb-609*::Tn*10 supD501*	D. Biek
TT7612	*zbf-98*::Tn*10 supE20*	D. Biek
TT7614	*zde-96*::Tn*10 supF30*	D. Biek
TT7674	*pncA212*::Mu d1-8	
TT7688	*hisC527 leuA414 zxx-1071*::Mu d1-8	
TT7692	*hisD9953*::Mu d1-8	
TT7963	*putP1017*::Mu d1	S. Maloy
TT8046	*proAB47 pyrB64* (F'128 *zzf-1066*::Mu d1-8)	D. Biek
TT8353	LT2 (pLP103-6-3) (Amp^r Mu A^+ B^+)	
TT8388	*zeb-609*::Tn*10 recA1*(F'128 *zzf-1066*::Mu d1-8)	
TT9749	*hsiD1299*::Mu d2-8[b]	
E. coli		
PP1073	*thi-8 galK1381* HfrH (pLP103-6-3) (Amp^r Mu A^+ B^+)	P. van de Putte
KL228	*thi-1 leu-6 gal-6 lacY1* or *lacZ4 supE44*	CGCS[c]
TR6547	*supD metF*(Am) *eda-50 ΔlacU169 rpsL136 thi-1 ara-14 mtl-1 xyl-5 tsx-78 tonA31*	
TR6548	*metF*(Am) *eda-50 ΔlacU169 rpsL136 thi-1 ara-14 mtl-1 xyl-5 tsx-78 tonA31*	
TT9812	Same as TR6547 with *srl*::Tn*10 recA1*	
TT9813	Same as TR6548 with *srl*::Tn*10 recA1*	
TT9828	Same as TT9813 with (F'128 *zzf-1066*::Mu d1-8)	
TT9894	Same as TR6547 with a Mu *c*62ts Mu d1-8 dilysogen	
TT9895	Same as TT9812 with a Mu *c*62ts Mu d1-8 dilysogen	
TT9897	Same as TR6548 with *thr*::Mu d1-8	

[a] Unless indicated, all strains were constructed during the course of this work.
[b] *hsi* is an extension of the *his* genetic nomenclature (see the text).
[c] Coli Genetic Stock Center, Yale University, New Haven, Conn.

Generation of a Mu d1 phage with a more complete transposition defect. Although the Mu d1-6 lysogen in a background lacking any suppressor mutation grew well at 42°C, it transposed at an unacceptably high frequency with increasing temperature (Table 2). To obtain more stable derivatives of Mu d1-6, advantage was taken of a growth property of Mu d1-6 lysogens. The Mu d1-6 lysogens grow very poorly at 43 to 45°C in nutrient agar, giving rise to faster growing revertants. Fifty independent temperature-resistant

TABLE 2. Mu d transduction in suppressor and nonsuppressor strains at various temperatures

Donor[a]	Temp (°C)	Suppressor in recipient strain	Frequency of analog-resistant transductants	% Inheritance of Mu d by transposition[b]
a. Mu dl	30	None	0/280	>99
b. Mu dl	37	None	0/224	>99
c. Mu dl	30	*supE*	0/219	>99
d. Mu dl	37	*supE*	0/88	>98
e. Mu dl-6	30	None	133/232	43
f. Mu dl-6	37	None	80/175	54
g. Mu dl-6	42	None	13/88	85
h. Mu dl-6	44	None	6/75[c]	92
i. Mu dl-6	30	*supE*	0/272	>99
j. Mu dl-6	37	*supE*	0/141	>99
k. Mu dl-6	42	*supE*	0/154[c]	>99
m. Mu dl-8	30	None	100/100	<1
n. Mu dl-8	44	None	65/65	<2
o. Mu dl-8	30	*supD*	295/3,990	93
p. Mu dl-8	30	*supE*	259/2,327	89
q. Mu dl-8	30	*supF*	207/3,185	94

[a] The donor strains used were TT7232 (Mu dl), TT7575 (Mu dl-6), and TT7674 (Mu dl-8). The recipients used were DB7136 (no suppressor), TR4299 (*supE*, lines c,d,i,j, and k), TT7610 (*supD*), TT7612 (*supE*, line p), and TT7614 (*supF*).

[b] The percent transposition represents the percentage of ampicillin-resistant transductants in which Mu d transposed to a new location on the chromosome as opposed to inheritance of Mu d by homologous recombination.

[c] Colonies grew very poorly.

revertants of Mu dl-6 were selected at 44°C and checked for their ability to transpose in a *sup*$^+$ (no suppressor) strain at 44°C. These temperature-resistant revertants fell into three major classes. When P22 grown on class 1 mutants was used as donor, 80% of the Amp^r transductants were due to transposition. When P22 grown on class 2 mutants was used as donor, 25 to 50% of the Amp^r transductants were due to transposition. When P22 grown on class 3 mutants was used as donor, less than 10% of the Amp^r transductants were due to transposition. One of the class 3 revertants showed no transposition in initial tests and was characterized further. This mutant was designated Mu dl-8.

Effects of amber suppressors on transposition ability of Mu dl-8. Transposition was assayed by transduction (simple transposition under zygotic induction conditions) and by transposition from one chromosomal site to another (replicative transposition). P22 grown on a *pncA*::Mu dl-8 mutant (6-aminonicotinamide resistant) was used to transduce a wild-type recipient to Amp^r and the percentage of 6-aminonicotinamide-sensitive (transposition) transductants was scored. Transposition during cell growth was scored by using four independent Lac$^-$ insertions of Mu dl-8. Mu d insertions are Lac$^-$ when inserted in an untranslated region of the chromosome or in a translated region in the orientation that does not transcribe the *lac* operon. Reversion of these mutants to Lac$^+$ is assumed to occur primarily by transposition to new sites; therefore, the reversion frequency of Lac$^-$ Mu d insertions to Lac$^+$ is an indication of transposition ability. (The validity of the assumption underlying this test was confirmed by subsequent experiments.)

Mu dl-8 insertions transduced into a strain lacking a suppressor mutation were inherited only by homologous recombination. This was true at both 30 and 44°C (Table 2, lines m and n). However, Mu dl-8 was inherited primarily by transposition in amber suppressor strains (89 to 94% of the Amp^r transductants) (Table 2, lines o, p, and q). To determine the low, residual transposition ability of Mu dl-8 in a strain lacking any suppressor mutation, P22 was grown on a *hisD*::Mu dl-8 insertion and used as donor to transduce LT2 to Amp^r at 30, 37, and 42°C. The Amp^r transductants were then screened for His$^+$ prototrophic types, which arose when Mu dl-8 transposed after transduction. Under these zygotic induction conditions and in the absence of a suppressor, only 0.2 to 0.3% of the Amp^r transductants obtained were His$^+$. Ten of the His$^+$ transductants obtained at 30°C were screened for preexisting duplications of the *his* region in the recipient (1). This was done by screening the Amp^r His$^+$ transductants for the ability to segregate Amp^r His$^-$ and Amp^s His$^+$ colonies after nonselective growth conditions. One of these was found to carry a preexisting duplication of the *his* region (a *hisD*::Mu dl-8 recombinant in such a recipient would appear as a transposition event [His$^+$] due to the presence of a second, intact *his* operon). Therefore, a majority of the His$^+$ transductants obtained in these crosses were presumably due to transposition. All other transductants were His$^-$ and arose by homologous recombination events involving the transduced fragment (Table 3).

To measure effects of amber suppressors on transposition of Mu dl-6 and Mu dl-8, we isolated Lac$^-$ Mu dl-6 and Mu dl-8 insertion mutations as described above, and the reversion frequencies to Lac$^+$ at various temperatures were determined. For Lac$^-$ insertions of the parental Mu dl phage, revertants arose most commonly by transposition to a secondary site that provided a promoter for *lac* operon expression. Lac$^-$ Mu dl-6 insertions in a *sup*$^+$ (no suppressor) strain were very stable at 30°C, reverting to Lac$^+$ at a frequency of 10^{-8} to 10^{-9} (Table 4, line a). When tested at 42°C, these same strains reverted at a frequency of 10^{-3} to 10^{-4} (Table 4, line b), which is similar to the reversion frequency of Lac$^-$ Mu dl-6 insertions in a *supE* strain at 30°C (Table 4, line c). The reversion frequency of 10^{-3} to 10^{-4} was the same as that reported for the original Mu dl Lac$^-$ insertions reverting to Lac$^+$ at 30°C (22).

TABLE 3. Zygotic induction of Mu dl-8 in wild-type (no suppressor) strain LT2

Temp (°C)	No. of Amp^r transductants that were His$^+$ (%)
30	18/6,000 (0.3)
37	8/3,000 (0.3)
42	1/563 (0.2)

[a] In all crosses the donor strain was TT7692 (*hisD9953*::Mu dl-8).

TABLE 4. Reversion of Lac$^-$ Mu d insertions to Lac$^+$

Insertion transposon	Suppressor	Temp (°C)	Frequency of Lac$^+$ revertants[a]
a. Mu dl-6	None	30	$<5 \times 10^{-9}$–20×10^{-9}
b. Mu dl-6	None	42	3×10^{-4}–5×10^{-4}
c. Mu dl-6	*supE*	30	3×10^{-4}–20×10^{-4}
d. Mu dl-8	None	30	$<5 \times 10^{-9}$–4×10^{-9}
e. Mu dl-8	None	37	$<5 \times 10^{-9}$–30×10^{-9}
f. Mu dl-8	None	42	6×10^{-8}–300×10^{-8}
g. Mu dl-8	*supE*	30	5×10^{-5}–100×10^{-5}
h. Mu dl-8	*supE*	37	5×10^{-3}–10×10^{-3}

[a] The frequencies in each case represent the range in the reversion frequency for four different Mu dl-6 or Mu dl-8 lysogens isolated in strain DB7155 (used in lines c,g, and h) and transduced into an isogenic suppressor-free strain, DB7136 (used in lines a,b,d,e, and f).

When the same experiment was performed with various Lac$^-$ Mu d1-8 insertions a different result was observed. In a strain with no suppressor, the Lac$^-$ Mu d1-8 insertions were very stable, reverting to Lac$^+$ at frequencies of 10^{-7} to less than 10^{-9} at 30 and 37°C (Table 4, lines d and e). At 42°C, there was a relatively slight increase in the reversion frequency to 10^{-6} to 10^{-7} (Table 4, line f). When Mu d1-8 was tested in *supE* strains at 30°C, the result was the same as with Mu d1-6, reversion at frequencies of 10^{-3} to 10^{-4} (Table 4, line g). At 37°C, this frequency was increased 10- to 100-fold (Table 4, line h). This increase with temperature is expected if the residual transposition functions are still under negative control of the temperature-sensitive repressor protein.

Complementation of the Mu d1-8 transposition defect by Mu *A* and *B* genes. Plasmid pLP103-6-3, constructed by P. van de Putte and co-workers, carries and expresses the Mu *A* and *B* gene products (30). However, these genes are not expressed at high enough levels to induce a Mu prophage (P. van de Putte, personal communication). This plasmid was introduced into the wild-type *Salmonella* LT2 strain, which carries no amber suppressor mutation. P22 phage grown on a Lac$^-$ Mu d1-8 insertion was used to transduce this strain to Lac$^+$. To generate a Lac$^+$ transductant, the donor Mu d1-8 must transpose to a new site. The transposition defect of Mu d1-8 was corrected in recipients which carry the plasmid-borne Mu *A* and *B* genes since the plasmid restored the ability of Mu d1-8 to generate Lac$^+$ transductants (Table 5). This result suggests that the mutations which eliminate Mu d1-8 transposition are in one or both of the *A* and *B* genes of Mu.

Isolation of random Mu d1-8 insertions. Mu d1-8 transductants into a suppressor-carrying strain were screened for auxotrophy resulting from insertions into biosynthetic genes. As discussed above, Mu d1-8 is inherited by transposition in amber suppressor strains at frequencies of 89 to 94% compared with inheritance by homologous recombination. To eliminate recombinational inheritance of Mu d1-8 and to have all transductants be the result of transposition, a strain was constructed with a Mu d1-8 insertion in an *E. coli* F′ strain (F′128) (Don Biek, unpublished results). This strain was used as donor in a P22-mediated cross with a suppressor-carrying recipient strain. The lack of homology between *E. coli* and *S. typhimurium* prevented the donor insertion in this cross from being inherited by standard recombination. A series of new Mu d1-8 insertion mutations was isolated in this way. The transductants obtained included auxotrophs at a frequency of ca. 2% of the total. Insertions in a wide variety of chromosomal genes resulted in various auxotrophic requirements (Table 6). This suggests that Mu d1-8 transposed into new sites in a suppressor-carrying strain as randomly as does Mu d1. In addition, Mu d1-8 insertions resulting in the inability to utilize various carbon sources have also been isolated (data not shown).

TABLE 5. Complementation of Mu d1-8 transposition by a plasmid encoding bacteriophage Mu *A* and *B* gene functions[a]

Relative concn of transducing phage	No. of Lac$^+$ transductants with recipient:	
	LT2	LT2/Mu A^+ B^+
100	1	>10^4
10	0	1,500
1	0	133

[a] The donor strain used in all crosses was TT7688 (Mu d1-8 Lac$^-$). LT2 is the wild-type *S. typhimurium* strain and LT2/Mu A^+ B^+ is strain TT8353.

TABLE 6. Auxotrophic Mu d1-8 insertions isolated in TT7610 after mutagenesis by Mu d1-8[a]

Auxotrophic requirement	No. isolated
Arginine	19
Adenine	6
Adenine or guanine[b]	52
Aromatic amino acids	23
Biotin	15
Cysteine	17
Cysteine or methionine	2
Glutamate	4
Glutamate or aspartate	7
Glutamate or glutamine	2
Glutamate or aspartate or glutamine	7
Glutamine	7
Guanine	5
Histidine	67
Isoleucine	2
Isoleucine + valine	14
Leucine	15
Lipoic acid	1
Lysine	3
Methionine	15
Methionine or cobalamin	19
Nicotinamide	20
Phenylalanine	10
Proline	5
Serine	2
Thiamine	9
Threonine	14
Tryptophan	35
Tyrosine	4
Uracil	43
Uracil + arginine	16
Valine	1
Unknown	12

[a] Isolated as auxotrophs among Amp^r transductants obtained in crosses with strain TT7674 (*pncA212*::Mu d1-8) or TT7688 (*hisC527 leuA414 zxx-1071*::Mu d1-8) as donor.

[b] Thiamine was included in media containing adenine or guanine.

Use of Mu d1-8 to select for mutants resulting in constitutive expression of the histidine biosynthetic operon. One of the main uses of Mu d fusions is to provide a simple means of isolating regulatory mutants by using Lac selection techniques. We selected for increased Lac expression of a *his*::Mu d1-8 insertion mutant to demonstrate that Mu d1-8 is stable enough to use directly for selection of constitutive mutants in the *his* operon. If regulatory mutants can be obtained with Mu d1-8 in *his*, then similar methods could presumably be used to obtain regulatory mutants for any operon.

A *his*::Mu d1-8 insertion, isolated as described above, was shown by complementation and deletion mapping experiments to be in the *hisD* gene (data not shown). This insertion mutant formed light-blue colonies when grown on minimal medium containing histidine and the chromogenic *lacZ* substrate X-gal and dark-blue colonies when starved for histidine. A 0.1-ml portion of an overnight culture of this strain was plated onto a minimal medium plate containing X-gal, and a crystal of histidine was added to the center. After overnight incubation, growth around the crystal occurred which was light blue near the crystal and dark blue along the edge of growth. This suggested that the insertion places the *lac* operon under control of the *his* promoter. When plated on lactose as sole carbon source, this strain grew slowly and

TABLE 7. Relative transposition frequency of Mu d2-8 in wild-type strain LT2 and amber suppressor strains

Recipient strain[a]	Suppressor	No. of Ap^r transductants scored	Temp (°C)	No. *his*$^+$ (%)
LT2	None	~7,000	30	49 (0.7)
		~5,000	37	69 (1.4)
		2,763	42	55 (2.0)
TT7610	*supD*	3,238	30	2,620 (81)
TT7612	*supE*	2,838	30	2,033 (72)
TT7614	*supF*	3,225	30	2,413 (75)

[a] In all crosses the donor strain was TT9749 (*hsiD1299*::Mu d2-8).

gave rise to faster-growing colonies. Four of these potential *his* constitutive colonies were selected from each of 11 independent cultures of the *hisD*::Mu d1-8 strain.

To remove the Mu d insertion and retain any possible regulatory mutation, these mutants were transduced to *his*$^+$ by using phage grown on a *his* promoter operator deletion mutant which still had an intact *hisD* gene. This cross would yield transductants that lost the Mu d1-8 insertion but retained the *his* control region of the fast-growing fusion strain. They would, of course, also retain any regulatory mutants not linked to the *his* operon (27). Four of the 11 cultures yielded *his*$^+$ transductants with a wrinkled colony morphology. The wrinkled colony phenotype is indicative of constitutive expression of the histidine operon (23). All of the *his*$^+$ transductants became sensitive to ampicillin, suggesting that the original *hisD*::Mu d1-8 insertion was the only copy of Mu d1-8 in the cell and none of the Lac$^+$ revertants were due to a Mu d1-8 transposition that places the lac operon under the control of a stronger promoter. The faster-growing derivatives which did not show a wrinkled colony morphology were probably low-level constitutive mutants that caused only small increases in *his* transcription, not enough to affect colony morphology.

Characterization of Mu d2-8 (Tpn[Am] Amp Lac *c*62ts), a transposition-defective derivative of Mu d2. The mutations which result in the conditional stability of Mu d1-8 were moved onto the protein fusion phage described by Casadaban and Chou, Mu d2 (9). This derivative, termed Mu d2-8, was constructed by using Mu d1-8 and Mu d2 in a transduction cross that forced the two inserted Mu d elements to recombine and generate a chromosomal duplication. These crosses are described in detail elsewhere (K. Hughes and J. Roth, submitted for publication). We characterized Mu d2-8 with respect to transposition in suppressor-carrying and suppressor-free strains.

The frequencies of inheritance of Mu d2-8 by transposition or homologous recombination are given in Table 7. In all cases the donor Mu d2-8 insertion was in the *hisD* gene. Amp^r transductants that were His$^+$ did not inherit the donor *hisD*::Mu d2-8 insertion phenotype. When introduced into a wild-type recipient, the background inheritance of Mu d2-8 by transposition varied from 0.7 to 2% of the total transductants. When introduced into suppressor-carrying recipients, Mu d2-8 was inherited by transposition between 72 and 81% of the time, depending on which amber suppressor was used. These results demonstrate that Mu d2-8 behaves similarly to Mu d1-8 as a transposition-defective Mu d derivative which is suppressed in the presence of amber suppressor mutations.

To effectively use Mu d2-8 for fusions, it was necessary to eliminate background recombination of the Mu d2-8 when transduced into suppressor-carrying recipients. The same method employed for eliminating the background recombination of Mu d1-8 was used. A strain was isolated which carries a Mu d2-8 insertion in an *E. coli* F prime, F'152 (Hughes and Roth, unpublished data). When P22 grown on this strain was used as donor into a suppressor-carrying recipient, 100% of the Amp^r transductants occurred by transposition of Mu d2-8 to new sites throughout the chromosome. The lack of homology between *E. coli* and *S. typhimurium* prevented the donor insertion in such crosses from being inherited by standard recombination.

The difference in relative transposition frequencies between Mu d1-8 (89 to 94%; Table 2, lines o, p, and q) and Mu d2-8 (72 to 81%; Table 7) may be due to the difference in the amount of Mu DNA sequence at the end before the *lac* operon. The ends of Mu are essential for Mu d transposition. In Mu d2-8, the amount of Mu sequence at the fusion is less than in Mu d1-8 (9). The shorter Mu sequence at the fusion end of Mu d2-8 may result in the slightly lower frequency of transposition events observed. We also noticed that Mu d2-8 insertion mutants in strains carrying an amber suppressor were not as temperature sensitive as similar Mu d1-8 insertion mutants. This result may also have been due to a lower amount of Mu DNA sequence at the fusion end of Mu d2-8, affecting the ability of insertions to undergo replicative transposition.

Isolation of Mu d1-8 fusions in *E. coli*. We found the Mu d1-8 phage useful for isolation of stable fusions in *E. coli* as well as in *S. typhimurium*. A Mu lysate was prepared on a Mu-Mu d1-8 dilysogen and was used to transduce a suppressor-carrying *E. coli* strain (TR6547) to Amp^r. Approximately 3% of the total Amp^r transductants were auxotrophs, suggesting that the Mu d1-8 was packaged by the Mu helper and transposed into the recipient by zygotic induction. A *thr*::Mu d1-8 insertion was isolated from the auxotrophic transductants and transduced into a wild-type strain. Phage P1 was then grown on this strain and used to transduce *E. coli* recipients with and without amber suppressors. In a cross into a *supD* strain, TR6547, 49% (282/578) of the Amp^r transductants were recombinants that inherited the donor *thr*::Mu d1-8 insertion phenotype. When transduced into an isogenic *supD*$^+$ recipient, all of the Amp^r transductants (316/316) inherited the donor *thr*::Mu d1-8 insertion. When the same experiment was done with a *supE* recipient, KL228, only 20% (173/849) of the Amp^r transductants inherited the donor *thr*::Mu d1-8 insertion. Thus, Mu d1-8 transposed by zygotic induction into *E. coli* amber suppressor strains with either Mu or P1 as vector, but not into the strain without an amber suppressor.

DISCUSSION

We have constructed a derivative of the Mu d1 phage described by Casadaban and Cohen (10) which is conditionally defective in transposition. The methods used to isolate a transposition-defective derivative of Mu may be generally useful in the study of Mu phage transposition. The main feature of these methods is the use of Mu d as a transposon rather than a phage. By doing this one can separate the transposition activity of Mu from the rest of Mu physiology. In addition, by use of the selectable phenotypes of Mu d1, as well as those of its derivatives, one can sensitively follow Mu d transposition even when rare.

Three criteria were used to demonstrate the transposition defect of Mu d1-8 and the correction of this defect by amber suppressors. These criteria were as follows. (i) An inserted

Mu d1-8 element was transduced (by phage P22) into a new recipient, and the relative frequencies of inheritance of the Mu d by recombination and by transposition were determined. (ii) The sensitivity of Mu d1-8 lysogens to killing by high temperature was checked. Since killing can be caused by the relief of repression of Mu transposition functions, this lethality is an indirect assay of transposition. (iii) The ability of a Lac^- Mu d1-8 insertion to transpose to a new site and thereby cause a Lac^+ revertant of the host strain was determined. By all these criteria, Mu d1-8 is defective in transposition in suppressor-free strains and regains transposition ability in the presence of an amber suppressor.

By using Mu d1-8 we were able to avoid the transposition-related problems associated with use of Mu d1. Two problems with Mu d1 are phage replicative transposition of lysogens grown at elevated temperatures and zygotic induction when the Mu d1 phage is introduced into a new host. These problems are avoided since Mu d1-8 is transposition-defective in strains which lack an amber suppressor mutation. In such strains, a Mu d1-8 prophage was not induced at elevated temperatures. When P22 grown on a Mu d1-8 lysogen was used to transduce Mu d1-8-encoded ampicillin resistance into a wild-type (no suppressor) recipient, almost all of the Amp^r transductants inherited the Mu d1-8 phage by homologous recombination. Transposition events occurred at a frequency of only 0.2 to 0.3% of the total Amp^r transductants. When a similar transduction was done with P22 grown on the nonmutant Mu d1 lysogen, greater than 99.9% of the Amp^r transductants inherited the Mu d1 phage that had transposed. Once inserted, the Mu d1 lysogens underwent transposition-related rearrangements at the permissive temperature (30°C) at frequencies of 10^{-3} to 10^{-4}. At 30°C, Mu d1-8 lysogens underwent similar rearrangements at frequencies of less than 5×10^{-9} to 4×10^{-9}. This is about the frequency with which one would expect rearrangements to occur in the absence of transposition functions. Finally, we also showed Mu d1-8 to be useful in making stable fusions in *E. coli* as well as in *S. typhimurium*.

The stability of the phages described here (Mu d1-8 and Mu d2-8) makes them particularly attractive for use in the study of gene regulation. The very low frequency of transposition permits simple selection of regulatory mutations without prior stabilization of the Mu d insertion. Also, the *lac* fusions can be transferred into any new genetic background without zygotic induction of the prophage. Thus, the phages facilitate construction of isogenic strains containing the *lac* fusions which can be grown and assayed at high temperature. An additional use of these stabilized phages is in the directed construction of chromosomal deletions and duplications; these methods will be described elsewhere (Hughes and Roth, submitted for publication).

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