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Genetics

Isolation of point mutations in bacteriophage Mu attachment regions cloned in a λ::mini-Mu phage

(transposable elements/site-specific recombination)

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ABSTRACT **Twenty-one derivatives of a λ::mini-Mu phage containing point mutations in the Mu attachment regions were isolated after *mutD* mutagenesis and selection for relief from Mu-specific replicative interference of λ growth. DNA sequence analysis revealed that the single left-end mutant had suffered a T → C transition at position 1 of the Mu sequence, while the remaining 20 right-end mutants contained single base-pair insertions or deletions within the terminal 19 base pairs. A genetic assay showed that the right-end mutations revealed by sequencing were necessary for relief of the replicative inhibition of λ growth. The properties of these mutants suggest that the terminal 2-base-pair and subterminal 8-base-pair inverted repeats are important for Mu-specific replicative transposition.**

The temperate bacteriophage Mu (1), which depends upon transposition for replication of its genome, requires the protein products of genes *A* and *B* and sites at each end of Mu DNA for the replicative transposition process (refs. 2 and 3; A. C. Glasgow, J. L. Miller, and M.M.H., unpublished results). Transposable genetic elements characteristically contain short regions of DNA of similar sequence at each end in inverted orientation. In general, these short inverted repeats range in length from 9 to 40 base pairs (bp) and are nearly perfectly homologous (4). Mu, on the other hand, has terminal inverted repeats of only 2 bp, and 8-bp subterminal inverted repeats beginning 12 and 21 bp from the right and left ends, respectively (5). Analysis of nuclease BAL 31-generated deletions showed that ≈160 bp of the left end and ≈50 bp of the right end were the minimal attachment regions necessary for efficient transposition of mini-Mu DNA (3). These regions correlate strongly with sites to which the Mu A protein binds *in vitro* (6).

Growth of λ::mini-Mu phages, λ phages containing the ends of Mu DNA cloned in their normal relative orientation, is inhibited in a Mu-specific reaction (7, 8). This λ-growth inhibition requires the products of genes *A* and *B* and the Mu attachment sites and is a consequence of Mu-specific DNA replication within the λ genome (A. C. Glasgow, J. L. Miller, and M.M.H., unpublished data). When the Mu A and B proteins are provided in *trans*, this property forms the basis of a direct selection for phages with mutations in the attachment regions of Mu. This paper describes the isolation and initial characterization of phages with point mutations in the minimal attachment regions of Mu.

MATERIALS AND METHODS

Bacterial and Bacteriophage Strains. The *Escherichia coli* K-12 strains used are listed in Table 1. The *hip157* allele from strain MH5792 was transferred to strain KD1067 to generate strain MH4389 by P1 *cm1 clr100* transduction, selecting for tetracycline resistance conferred by the linked Tn*10*; the *himA42 zdh201*::Tn*10* alleles from MH4386 were similarly cotransduced into strain MH4392 to generate strain MH4398. Strain MH7313 was constructed by first generating a MudII301(Ap,*lac*) lysogen of MH349 as described (14). Lac$^+$ lysogens were mated with MH5500 at a donor to recipient ratio of 10:1. Ampicillin-resistant (Amp^R) colonies were screened for the presence of an F factor by cross-streaking (11) against phage T7, which will not grow on male strains (15). Male strains were then plated at 5×10^4 cells per plate on lactose/tetrazolium plates (16) to detect Lac$^-$ homogenotes. Red colonies were picked and purified, and one of several with a stable Lac$^-$ phenotype, MH7308, was transduced to tetracycline resistance using a lysate of P1*vir* grown on MH4386. One such transductant, MH7313, grew at 42°C, indicating that it carried the linked *himA42* mutation that inhibits growth of the lysogenic MudII phage (17). The *him*$^+$ parent of MH7313, MH7308, was temperature sensitive, Mu-immune, and, unlike MH7313, able to transfer the Amp^R marker in a conjugation assay (18) due to transposition of the MudII prophage. Strain MH4380 was constructed by transformation of strain QD5003 with plasmid pLP103-6 (19).

The λ::mini-Mu phage Δ346 (A. C. Glasgow, J. L. Miller, and M.M.H., unpublished data) is a deleted derivative of phage λ318*imm*434, which in turn is an *imm*434 derivative of phage N318, a λ::mini-Mu phage containing the left and right ends of Mu in their normal relative orientation (7). Phages with an "M" prefix are mutant derivatives of Δ346.

Isolation of Mutant λ::mini-Mu Phages. Strain MH4389 was grown in LB (Luria broth) containing 5 mM $MgSO_4$ and 0.2% maltose to 2×10^8 cells per ml. After centrifugation and resuspension in 1/5 vol of the same medium, 1 ml of cells was infected with 1 ml of a lysate of Δ346 (4×10^9 phage) for 15 min at 37°C. The mixture was diluted into 50 ml of LB containing 2 mM $MgSO_4$, distributed into 50 tubes, and grown with shaking at 37°C for 1 hr. The cultures were chilled and treated with chloroform, and dilutions were adsorbed to strain MH4380, plated on TCMG agar (7) with 2.5 ml of LB soft agar, and incubated overnight at 37°C to select for mutants. Each mutant studied was from a separate culture.

Media and Phage Methods. LB agar, LB broth, TCMG, and soft agar were described (7). LB/Amp plates contained 50 μg of ampicillin per ml. Large and small lysates of λ::mini-Mu phages were prepared by infection of strain K750 as de-

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Abbreviations: bp, base pair(s); kb, kilobase(s); EOP, efficiency of plating; Amp^R, ampicillin resistant.

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Table 1. Bacterial strains

Strain	Genotype	Source or ref.
K750	F+ *supF mel pro himA42*	7
KD1067	F− *argE his mutD5*	9
MAL315	F− *araD139* Δ(*lac-proA,B*)*XIII rpsL* Δ(*ara-leu*)*7697 mal*(*K* or *T*)::Mu *cts62* MudII301(Ap,*lac*)	10
MH349	F− Δ(*lac-pro*) *trp*Δ*ED24 rpsL*	M.M.H. lab
MH4380	F+ *supF mel pro*/pLP103-6	This work
MH4386	*gal rpsL himA42 zdh201*::Tn*10*	11
MH4389	F− *argE his mutD5 hip157* Tn*10-1230*	This work
MH4392	F+ *araD* λ*imm*434	RS54 λ*imm*434
MH4398	F+ *araD himA42 zdh201*::Tn*10* λ*imm*434	This work
MH5500	F′ *lac*Δ*FMS37*/ *thi lac*Δ*X74*	12
MH5792	HfrH *galT*::λΔ(*int-FII*) *hip157* Tn*10-1230*	11
MH7308	F′ *lac*Δ*FMS37*/ Δ(*lac-pro*) *trp*Δ*ED24 rpsL* MudII301-1 (Ap,*lac*Δ*FMS37*)	This work
MH7313	F′ *lac*Δ*FMS37*/ Δ(*lac-pro*) *trp*Δ*ED24 rpsL* MudII301-1(Ap,*lac*Δ*FMS37*) *himA42 zdh201*::Tn*10*	This work
QD5003	F+ *supF mel pro*	7
RS54	F+ *araD*	13

scribed (20). MudII301(Ap,*lac*) lysates were prepared by heat induction of strain MAL315 as described (10). P1 lysates were prepared and transductions were performed essentially as described by Miller (16).

Recombinant DNA Methods. Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs, Boehringer Mannheim, and Promega Biotec (Madison, WI) and used according to the manufacturers' suggested conditions or those described by Maniatis *et al.* (21). Transformations were performed as described (22, 23). Agarose electrophoresis and electroelution of DNA fragments from agarose gel slices were performed essentially as described (21). Eluted fragments were purified by adsorption to and elution from an Elutip-d column (Schleicher & Schuell) according to the manufacturer's directions, followed by ethanol precipitation (21).

Cloning of Mu DNA from λ::mini-Mu Phages. Phage DNA was isolated from particles purified from 4-liter lysates as described (20). The DNA was digested with *Eco*RI and *Hin*dIII and mixed with *Eco*RI/*Hin*dIII-digested pBR322 DNA (24) at a target to vector end ratio of 3:1 (1 μg of total DNA). After ligation and transformation into strain K750, Amp^R transformants were isolated on LB/Amp plates. Plasmid DNA was isolated by the method of Birnboim and Doly (25), and transformants were screened for the presence of Mu DNA fragments by digestion of the plasmid DNA with *Eco*RI and *Hin*dIII followed by electrophoresis through agarose gels.

Marker Rescue Assay. Strains containing plasmids with mini-Mu DNA fragments were grown to 10^9 cells per ml in LB containing 2.5 mM $MgSO_4$, 0.2% maltose, and 50 μg of ampicillin per ml. After 1:200 dilution into LB containing 2.5 mM $MgSO_4$, 0.1 ml of diluted culture was infected with Δ346 at multiplicities of infection of 0.06–0.2. After 15 min at 37°C, 0.2 ml of MH4380 at 10^9 cells per ml was added, and the mixture was plated in 2.5 ml of soft agar on TCMG plates. Plaques were counted after overnight incubation at 37°C.

DNA Sequencing. For DNA sequencing, plasmid DNA was isolated by the method of Humphreys *et al.* (26). Plasmids were digested with *Hin*dIII and either *Eco*RI or *Eco*RV, and the 1.15-kilobase (kb) fragment containing the left Mu attachment site or 1.13-kb fragment containing the terminal 480 bp of Mu DNA were isolated after electrophoresis through 0.8% agarose gels and electroelution. These fragments were used as substrates for Maxam and Gilbert DNA sequencing (27).

Preparation of Recombinant λ::mini-Mu Phages Carrying the Amp^R Gene from MudII301(Ap,*lac*) and Determination of Their Phenotype. Lysates of the λ::mini-Mus to be recombined with MudII301-1(Ap,*lac*Δ*FMS37*) were prepared by infection of MH7313. Strain MH4398 was infected with the resulting lysates at a multiplicity of infection of 2 and incubated for 15 min at 32°C. Nine volumes of LB containing 2.5 mM $MgSO_4$ were added, cells were grown for 4 hr at 32°C, and cultures were plated on LB/Amp plates. After growth at 32°C, Amp^R lysogens were purified, and the spontaneous phage released from exponentially growing cultures were harvested by treating the cultures with chloroform and centrifuging to remove debris. To score for the λ-growth inhibition phenotype, phages were spotted onto lawns of K750, QD5003, and MH4380. Inhibition-proficient (wild-type) phages gave zones of lysis only on K750; inhibition-defective (mutant) phages lysed all three strains. The resident λ*imm*434 prophage from MH4398 was not released due to the *himA* mutation (17). For efficiency of plating (EOP) assays and DNA isolation, released phage were purified on strain K750 prior to preparation of lysates by infection of K750.

RESULTS

Isolation and Preliminary Characterization of λ::mini-Mu Phages with Point Mutations Near the Mu Ends. The λ::mini-Mu phage Δ346 (Fig. 1) was mutagenized by growth on the *mutD* strain MH4389, and mutants were selected by plating the resulting phages on MH4380, a strain containing the plasmid pLP103-6 (19), which constitutively expresses early Mu genes including *ner*, *A*, and *B*. Because A and B products were provided in *trans*, the mutants were expected to have defects in the attachment sites. The 45 mutant phages isolated had EOPs of ≈1.0 on MH4380 as compared to strain K750 (*himA*), while the parent phage had an EOP of $<3 \times 10^{-3}$. Restriction analysis of DNA from these mutant phages showed that 24 had deletions removing one of the attachment sites while DNA from the remaining 21 mutant phages had *Eco*RI–*Hin*dIII restriction fragmentation patterns indistinguishable from the parent phage (D.L.L. and M.M.H., unpublished data).

Mu-containing *Eco*RI–*Hin*dIII restriction fragments from Δ346 and the apparent point mutant phages were inserted into pBR322 and transformed into strain K750. The 4.1-kb fragment (Fig. 1) could not be cloned, presumably due to expression of the *kil* gene (19). To determine which cloned fragments contained mutations, the ability of the parent phage Δ346 to acquire the mutations by recombination was assayed by marker rescue. The results (Table 2) demonstrate that for each mutant phage, one and only one cloned fragment

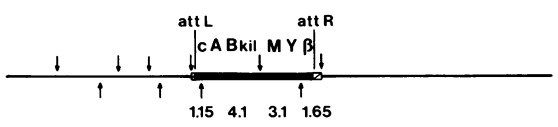

FIG. 1. Restriction map of the λ::mini-Mu phage Δ346, a derivative of λ318*imm*434. Downward and upward arrows represent *Eco*RI and *Hin*dIII restriction sites, respectively. The line represents λ DNA; the solid box represents Mu DNA; hatched boxes are host DNA flanking the Mu ends. Symbols above the line designate Mu sites and functions. Numbers below the line are the sizes (in kb) of *Eco*RI–*Hin*dIII fragments containing Mu DNA. The right and left arms of λ are on the left and right, respectively.

Table 2. Localization of mutations by marker rescue

Phage	Relative number of phage released		
	1.15 kb	1.65 kb	3.1 kb
M6	5.5 (2.9–9.4)	1.0 (0.8–1.3)	1.1 (0.9–1.4)
M10	1.2, 0.8, 1.3	11.4, 4.6	1.0, 0.9
M14	3.2, 1.6	9.7 (5.4–22.8)	3.2, 0.7
M19	1.8, 0.8	22.6, 6.7, 10.5	nc
M23	0.9, 1.1, 0.6	12.4 (7.5–19.0)	0.7 (0.2–0.9)
M25	1.8, 1.0	15.4, 6.0	0.7, 0.8
M29	1.7 (1.0–4.2)	10.0 (2.0–26.0)	1.2 (0.7–1.9)
M31	1.3 (0.8–1.6)	5.9 (3.3–7.8)	0.7 (0.1–0.9)
M33	1.3 (0.6–2.5)	4.2, 4.7, 4.7	0.8 (0.5–0.9)
M34	1.6, 1.4, 1.1	14.2 (6.7–34.0)	nc
M35	0.4, 1.3, 1.2	7.4, 7.2	0.5, 0.8, 0.6
M43	0.5, 1.5, 1.5	7.1, 9.8	1.0, 0.5
M47	2.3, 1.6, 0.9	12.3 (8.7–18.6)	0.5, 1.1, 0.8
M48	1.9, 0.8	14.4, 14.4, 7.8	1.2, 0.6

The relative number of phage released from bacteria harboring plasmids containing the indicated Mu DNA fragments was determined. The numbers in the table represent the fold increase in the number of plaque-forming units detected over background in multiple experiments. Background is defined as the mean titer of phage able to form plaques on MH4380 after infection by Δ346 of strains containing cloned 1.15-kb, 1.65-kb, and 3.1-kb Mu DNA fragments from Δ346. In each single experiment the three values used to compute the average were approximately equal. In different experiments, background varied from $0.4–2.2 \times 10^{-3}$ plaque-forming units per infecting phage. Multiple entries indicate that either a given clone was assayed more than once or multiple clones containing the same Mu DNA fragment were tested. When four or more assays were performed for a given fragment, the average and range (in parentheses) are given. nc, Not cloned. The results for M1, M2, M9, M26, M32, M44, and M50 were within the ranges observed for other mutants with the same base changes (see Fig. 3).

gave a positive marker rescue result; i.e., infection of a strain harboring a plasmid containing the cloned fragment resulted in increased release of phages able to form plaques on MH4380. Mutant M6 apparently contained a mutation in the 1.15-kb left-end fragment while the remaining 20 mutants had defects in the 1.65-kb right-end fragment. Thus, all DNA fragments giving marker rescue were from clones containing the left or right attachment site of Mu.

Sequence Changes in Mutant Phages Defective for λ-Growth Inhibition. DNA from the attachment regions of clones giving positive marker rescue results was sequenced using the strategies shown in Fig. 2. DNA from the parent phage Δ346 was sequenced using all the strategies shown except for strategy 9. Thus, a contiguous region of the left-end DNA extending from 140 bp left of *attL* to a point 641 bp into Mu was sequenced on at least one strand, and the sequence obtained agrees exactly with that published by Priess *et al.* (28). For the right end, at least one DNA strand from Δ346 was sequenced from *attR* 352 bp into Mu and 457 bp into the attached host DNA; both strands were sequenced for the terminal 343 bp of Mu and 258 bp of adjacent host DNA. The Mu DNA sequence agrees with that published by Groenen *et al.* (3) for the right-terminal 220 bp and with the sequence of Plasterk *et al.* (29) for positions 221–352 from the right attachment site.

The left-end mutant M6 was sequenced using strategies 1–5 (from 141 bp to the left of *attL* to 655 bp into Mu). The only mutation found was a T → C transition at position 1 of the Mu sequence (Fig. 3). DNA from all of the right-end mutants was sequenced using strategy 8; between 98 and 213 bp of Mu sequence was obtained for each mutant. All mutants contained single base-pair insertions or deletions within the terminal 19 bp of the Mu right end (Fig. 3). One of the cytosine residues at positions 7 or 8 was deleted in mutant M10. Other mutants were the result of deletion or insertion of a thymine

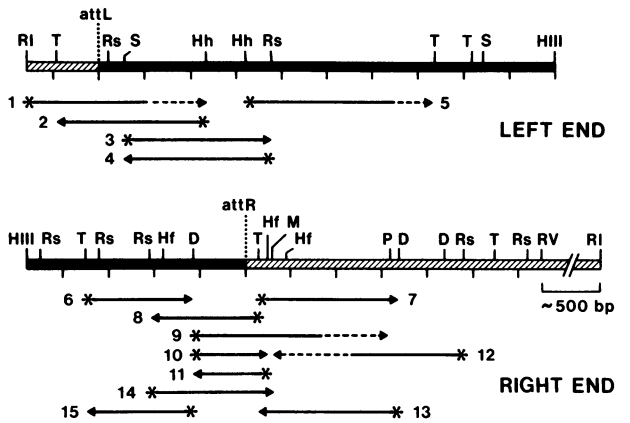

FIG. 2. Strategies used for Maxam–Gilbert sequencing of cloned λ::mini-Mu phage DNA fragments. Asterisks represent sites that were 5′ end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Arrow heads represent unlabeled fragment ends. The dashed portion of an arrow indicates the region of the fragment that was not sequenced. Vertical lines below the DNA are at 100-bp intervals. Restriction site abbreviations: D, *Dde* I; HIII, *Hin*dIII, Hf, *Hin*fI; Hh, *Hph* I; M, *Mbo* II; P, *Pvu* II; RI, *Eco*RI; RV, *Eco*RV; Rs, *Rsa* I; S, *Sau*3A, T, *Taq* I. Other conventions are as in Fig. 1.

residue within five consecutive thymine residues at positions 15–19 from the right end. Additional DNA was sequenced from mutants M10, M19, M23, M25, M43, and M48—representatives of each class of right-end mutant—using strategies 6–9, covering a segment of DNA extending from at least 256 bp into the host DNA and at least 339 bp into Mu. No additional mutations were found.

Mutations Found in the Right End of Mu Are Sufficient to Cause the Observed Phenotype. A marker rescue assay was used to show that the mutations at the right end revealed by sequencing were responsible for the growth-inhibition phenotype. The mutant phages were used to infect the *himA* strain MH7313, which is lysogenic for a derivative of the translation fusion phage MudII(Ap,*lac*) that contains only the terminal 117 bp of the 1.65-kb right-end fragment of Δ346 (10, 30). This 117 bp has been sequenced in all but five of the right-end mutants. Selection of Amp^R lysogens of MH4398 (*himA* λ*imm*434) containing λ::mini-Mu phages arising by *in vivo* recombination with the MudII prophage should yield phages in which all but the last 117 bp of the Mu DNA from the 1.65-kb fragment have been replaced by non-Mu DNA, as illustrated by the dashed lines in Fig. 4. If recombination

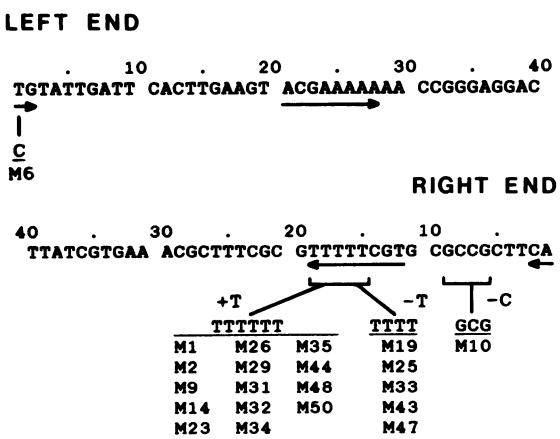

FIG. 3. Sequence changes in inhibition-defective mutants. Solid arrows below the sequence indicate inverted repeats, and numbers above the sequence indicate the distance in nucleotides from the Mu-host junction.

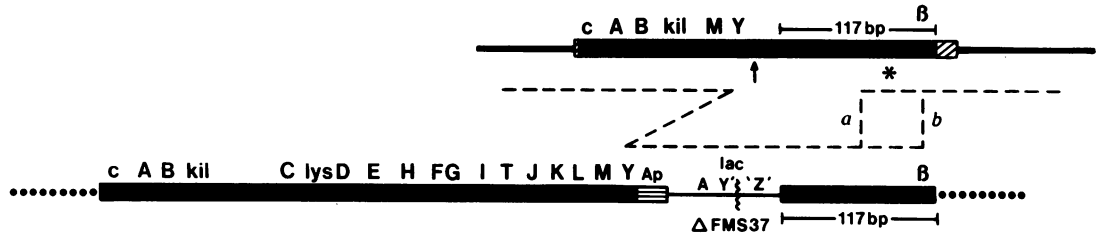

FIG. 4. Genetic strategy used to show that mutations preventing λ-growth inhibition are located within the last 117 bp of Mu DNA. At the top is a representation of a mutant λ::mini-Mu phage in which the Mu sequences (especially the last 117 bp) are exaggerated for clarity. At the bottom is a diagram of the MudII301-1(Ap,*lacΔFMS37*) prophage of strain MH7313. Conventions are the same as in Fig. 1 except that the dotted line represents *E. coli* chromosomal DNA, □ denotes the Amp^R determinant from Tn*3*, the thin line represents *lac* DNA, and the asterisk denotes a point mutation. The upward arrow represents the *Hin*dIII site at the junction between the 3.1-kb and 1.65-kb fragments of Δ346 and its mutants (see Fig. 1). This *Hin*dIII site is not present in MudI(Ap,*lac*) (30) or its derivative MudII301(Ap,*lac*), indicating that the only region of homology between the 1.65-kb fragment of Δ346 and MudII301-1 is at the terminal 117 bp of the Mu DNA. Recombinant λ::mini-Mu phages containing the Amp^R determinant were generated and scored for the λ-growth inhibition phenotype. If recombination occurred using path a, the mutant λ::mini-Mu would retain the mutation and remain inhibition defective. Recombination via path b would result in rescue of the wild-type allele from the prophage, and the recombinant phage would be inhibition proficient. Since λ can package only ≈53 kb of DNA (31), Amp^R phages resulting from recombination between the early regions of the two Mu genomes would not be viable. The FMS37 deletion, which removes 1.5–2 kb of the *lac* DNA (data not shown), was introduced into the prophage to ensure that the desired recombinants could be packaged.

followed path a, recombinant λ::mini-Mus would retain the mutation and would be defective for λ-growth inhibition (able to grow on Mu-permissive hosts). If recombination followed path b, recombinants would be inhibition proficient and unable to grow on Mu-permissive hosts.

Recombinant phages were isolated using MH7313-grown lysates of Δ346 and M10, M23, and M43—representatives of each class of right-end mutant—and scored for λ-growth inhibition (Table 3). Of 50 Amp^R lysogens of MH4398 resulting from infection with Δ346 lysates grown on MH7313, 30 released phage, all of which were inhibition proficient, as expected. When lysates of M10, M23, and M43, grown on MH7313, were used to generate Amp^R lysogens, both inhibition-proficient and inhibition-defective phages were released, suggesting that recombination utilized both paths a and b (Fig. 4). Recovery of both types of phages indicates that the mutation responsible for the difference in growth phenotype falls within the terminal 117 bp of Mu DNA. The only mutations in that region are those shown in Fig. 3.

Several control experiments using the Amp^R phages arising from the MudII recombination experiment showed that their properties were consistent with those expected from recombination. (*i*) The inhibition phenotype was confirmed in quantitative EOP assays using lysates prepared from each inhibition-proficient phage and eight inhibition-defective recombinant phages (four from M10 and two each from M23 and M43). (*ii*) DNA isolated from all but two of these phages had identical restriction fragmentation patterns consistent with the recombination pathways shown in Fig. 4 (data not shown). (The two exceptional phages, one defective and one proficient for inhibition, have not been further characterized.) (*iii*) The frequency of reversion to inhibition proficiency was found to occur at a frequency lower than that observed for the generation of inhibition-proficient phages by recombination. Four of the inhibition-defective Amp^R phages (two from M10 and one each from M23 and M43) were plaque-purified, grown on K750, and used to infect MH4398 selecting for Amp^R lysogens. Of 90, 47, and 44 lysogens generated from the M10, M23, and M43 derivatives, respectively, all released phage, none of which were inhibition proficient.

DISCUSSION

In this report we document the isolation of λ::mini-Mu phages with point mutations in the Mu attachment sites. The mutations described fall within regions known to be essential for Mu transposition (3) and to which the Mu transposase binds (6). In addition, these mutations cause a reduction in Mu-specific DNA replication and integration (R.P.B. and M.M.H., unpublished data).

Table 3. Properties of Amp^R λ::mini-Mu phages

Phage lysate*	Phage releasing lysogens/total lysogens†	Lysogens releasing Inh^+ phage/total phage-releasing lysogens‡
Δ346		
A	7/10	7/7
B	7/10	7/7
C	5/10	5/5
D	3/10	3/3
E	8/10	8/8
Total	30/50	30/30
M43		
A	1/4	1/1
B	4/18	2/4
C	2/10	1/2
D	5/10	5/5
E	1/20	1/1
Total	13/62	10/13
M10		
A	3/13	2/3
B	2/19	0/2
C	2/6	0/2
D	5/10	0/5
E–H	7/39	0/7
I–O	0/78	0/0
Total	19/165	2/19
M23		
A	6/10	0/6
B	3/10	3/3
C–G	0/44	0/0
Total	9/64	3/9

*Each entry (A–O) represents a lysate grown on MH7313 from an independent plaque and used to infect MH4398.

†Number of Amp^R lysogens of MH4398 releasing phage/total lysogens tested. The lysogens that failed to release viable phage probably contain prophages that arose by recombination between the early regions (*c*, *A*, and *B* genes) of the two Mu genomes illustrated in Fig. 4 and then suffered deletions that reduced the λ DNA length to a size able to be packaged. These deletions presumably removed functions essential for normal λ development.

‡Phenotype of released phage. Inh^+, inhibition-proficient phage unable to grow on Mu-permissive hosts, as assayed by spotting on bacterial lawns of K750, MH4380, and QD5003.

Unfortunately, only a small number of mutant classes were isolated. Of 21 point mutations, 19 were deletions or insertions of one thymine residue within a run of five consecutive thymine residues near the right Mu terminus. Sequences of consecutive residues tend to act as mutational "hotspots" (32), a likely explanation for the nonrandom distribution of mutations seen here. It is unclear whether the mutations were spontaneous or arose by *mutD* mutagenesis, since there was a less than 2-fold increase in the frequency of mutants after growth of the Δ346 phage on the *mutD* host (data not shown), and the types of mutations observed—transitions and frameshifts—are known to occur both spontaneously and after *mutD* mutagenesis (33, 34).

Sequence analysis of DNA from mutant phages suggests that the terminal 2-bp and subterminal 8-bp inverted repeats of Mu are important for replicative transposition. Twenty of the 21 mutant phages have mutations within these sequences. The remaining mutant M10 contains a 1-bp deletion that decreases the spacing between the two inverted repeats of the right end (Fig. 3). The importance of inverted repeat and flanking sequences has been amply demonstrated in other transposition systems. Point mutations within the last 13 bp of IS*10*-R substantially diminish transposition (35) as does a small insertion/deletion within one of the inverted repeats of Tn*3* (36). Similar analyses have shown that subterminal deletions extending into the short inverted repeats at the ends of IS*10*-R (35), IS*50* (37), and spleen necrosis virus (38) result in reduction of the integration frequency of the respective elements.

It is noteworthy that most of the λ::mini-Mu point mutations fall within the sequence $\overset{5'\ \text{ACGAAAA}\ 3'}{3'\ \text{TGCTTTT}\ 5'}$, a sequence also found in the attachment regions of Tn*3*, γδ, and IS*101*—transposable elements that, like Mu, generate 5-bp duplications upon insertion (4). This sequence is also found at positions 21–27 of the Mu left end and the seven consecutive adenine residues present (Fig. 3) should also serve as a mutational hotspot. That mutations were not recovered within the left-end sequence suggests that the two ends are functionally asymmetric or that the selection requires a substantially reduced efficiency of replicative transposition that cannot be accomplished by introducing single-site mutations into the left symmetry element. Since these sequences are subsets of sequences to which the Mu A protein binds (6), it is tempting to speculate that these mutations directly affect A protein binding. Alignment of the A protein recognition sites at the left and right ends shows that they are in different portions of the A binding domain. According to Craigie *et al.* (6), A protein binds more strongly to the leftmost A binding site (L1) than to the rightmost binding site (R1). Single-site mutations within L1 might, therefore, have less effect on A binding and thus on replicative transposition than similar mutations in R1.

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1. Toussaint, A. & Résibois, A. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 105–158.
2. Mizuuchi, K. (1983) *Cell* **35**, 785–794.
3. Groenen, M. A. M., Timmers, E. & van de Putte, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2087–2091.
4. Kleckner, N. (1981) *Annu. Rev. Genet.* **15**, 341–404.
5. Kahmann, R. & Kamp, D. (1979) *Nature (London)* **280**, 247–250.
6. Craigie, R., Mizuuchi, M. & Mizuuchi, K. (1984) *Cell* **39**, 387–394.
7. Schumm, J. W. & Howe, M. M. (1981) *Virology* **114**, 429–450.
8. Howe, M. M. & Schumm, J. W. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 1169–1177.
9. Degnen, G. E. & Cox, E. C. (1974) *J. Bacteriol.* **117**, 477–487.
10. Casadaban, M. J. & Chou, J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 535–539.
11. Ross, W., Shore, S. H. & Howe, M. M. (1986) *J. Bacteriol.*, in press.
12. Malamy, M. H. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 189–201.
13. Howe, M. M. (1973) *Virology* **54**, 93–101.
14. Csonka, L. N., Howe, M. M., Ingraham, J. L., Pierson, L. S., III, & Turnbough, C. L., Jr. (1981) *J. Bacteriol.* **145**, 299–305.
15. Morrison, T. G. & Malamy, M. H. (1971) *Nature (London) New. Biol.* **231**, 37–41.
16. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Miller, H. I. & Friedman, D. I. (1980) *Cell* **20**, 711–719.
18. Chaconas, G., Harshey, R. M., Sarvetnick, N. & Bukhari, A. I. (1981) *J. Mol. Biol.* **150**, 341–359.
19. van Leerdam, E., Karreman, C. & van de Putte, P. (1980) *Mol. Gen. Genet.* **179**, 185–189.
20. Howe, M. M., Schumm, J. W. & Taylor, A. L. (1979) *Virology* **92**, 108–124.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
22. Cohen, S. N., Chang, A. C. Y. & Hsu, L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110–2114.
23. Kushner, S. R. (1978) in *Genetic Engineering*, eds. Boyer, H. B & Nicosia, S. (Elsevier, Amsterdam), pp. 17–23.
24. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heynecker, H. L., Boyer, H. W., Crosa, J. H. & Falkow, S. (1977) *Gene* **2**, 95–113.
25. Birnboim, H. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523.
26. Humphreys, G. O., Willshaw, G. A. & Anderson, E. S. (1975) *Biochim. Biophys. Acta* **383**, 457–463.
27. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–559.
28. Priess, H., Kamp, D., Kahmann, R., Brauer, B. & Delius, H. (1982) *Mol. Gen. Genet.* **186**, 315–321.
29. Plasterk, R. H. A., Vollering, M., Brinkman, A. & van de Putte, P. (1984) *Cell* **36**, 186–196.
30. O'Connor, M. B. & Malamy, M. H. (1983) *J. Bacteriol.* **156**, 669–679.
31. Feiss, M., Fisher, R. A., Crayton, M. A. & Egner, C. (1977) *Virology* **77**, 281–293.
32. Pribnow, D., Sigurdson, D. C., Gold, L., Singer, B. S., Napoli, C., Brosius, J., Dull, T. J. & Noller, H. F. (1981) *J. Mol. Biol.* **149**, 337–376.
33. Cox, E. C. (1976) *Annu. Rev. Genet.* **10**, 135–156.
34. Lorenzetti, R., Cesareni, G. & Cortese, R. (1983) *Mol. Gen. Genet.* **192**, 515–516.
35. Way, J. C. & Kleckner, N. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3452–3456.
36. Gill, R. E., Heffron, F. & Falkow, S. (1979) *Nature (London)* **282**, 797–801.
37. Johnson, R. C. & Reznikoff, W. S. (1983) *Nature (London)* **304**, 280–282.
38. Panganiban, A. T. & Temin, H. M. (1983) *Nature (London)* **306**, 155–160.