Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6826–6830, September 1988
Genetics

Bacteriophage Mu sites required for transposition immunity

(plasmid/transduction/conjugation/deletion analysis/insertion)

ALDIS DARZINS, NANCY E. KENT, MARION S. BUCKWALTER, AND MALCOLM J. CASADABAN*

Department of Molecular Genetics and Cell Biology, The University of Chicago, 920 East 58th Street, Chicago, IL 60637

Communicated by Hewson Swift, May 23, 1988 (received for review February 26, 1988)

ABSTRACT **Plasmids with bacteriophage Mu sequences receive additional Mu insertions 20–700 times less frequently than plasmids without Mu sequences. The Mu sites required for this transposition immunity were mapped near each end, either of which was sufficient. The left site was between 127 and 203 base pairs from the left end, and the right site was between 22 and 93 base pairs from the right end. These sequences include the innermost but not the outermost of the three binding sites for the Mu A transposition protein at each end of Mu. Transposition immunity was cis-acting and independent of its location on a target plasmid. An additional copy of an immunity site reduced transposition a factor of 10 further. Transposition immunity was seen both during full phage lytic growth, with all the bacteriophage Mu genes, and during normal cellular growth, with a mini-Mu element containing only the Mu *c* and *ner* regulatory and *A* and *B* transposition genes.**

Mu is a temperate bacteriophage that replicates by a process that involves DNA transposition (1–4). Upon infection of a susceptible host, Mu DNA can integrate into the host DNA and either establish lysogeny or grow lytically as a phage with hundreds of transposition events. The high frequency with which Mu can transpose has made it a model system for genetic and biochemical analysis.

Transposition of Mu requires both phage- and host-encoded functions. The Mu A transposase protein is absolutely essential for transposition, while the B protein and the *arm* functions stimulate transposition 100-fold and 10-fold, respectively (5–7). The host-encoded HU protein is also required for the transposition reaction *in vitro* (8). Sequences required in cis for Mu transposition are located at both termini, within 163 base pairs (bp) of the left end and 52 bp of the right end (9). At each end Mu has many repeated sequences, including the terminal 2 bp that are inversely repeated and three nonidentical 30-bp sequences at each end for binding the Mu A transposase protein (10). The two Mu ends are not fully equivalent, although Mu elements can transpose at reduced frequencies with two right or two left ends, or with one end and a fortuitous sequence as the other end (11–14). Further, in the *in vitro* system Mu DNA precut at its 3′ ends transposes without regard to the ends being on the same molecule, with the right end being used 6 times more frequently than the left end (15).

Mu transposition *in vitro* is preferentially directed to plasmids that do not contain Mu sequences (8). During the course of this work Adzuma and Mizuuchi (16) have shown that this preference does not occur *in vitro* for the low level of transposition that is found without the Mu B protein, and Mogutov *et al.* (17) and Reyes *et al.* (18) have reported that Mu sequences in plasmids reduce the frequency of Mu transposition into those plasmids *in vivo*. Here we also report this and proceed to map the sites involved to a unique position within each Mu terminal region. We also demonstrate that no later Mu phage products are required.

MATERIALS AND METHODS

Bacterial Strains and Media. Strain MG1655.10 was derived from MG1655 (19) by introducing the *recA56* and Δ(*lac*) mutations with a series of four P1 transductions. MB1 is a Mu dII1678 (Ap^R) lysogen of MG1655.10 (20). Bacteria were grown with Luria–Bertani (LB) or M63 minimal glucose medium (21). For solid media, 1.5% agar was added. The antibiotic concentrations used were as follows: ampicillin (Ap), 25 μg/ml; carbenicillin (Cb), 500 μg/ml; chloramphenicol (Cm), 20 μg/ml; kanamycin (Km), 25 μg/ml; streptomycin (Sm), 100 μg/ml (for *rpsL* strains); and tetracycline (Tc), 20 μg/ml.

General DNA Methods and DNA Sequencing. Plasmid DNA was isolated by a modification of the procedure of Kupersztoch and Helinski (22). Small-scale plasmid DNA preparations, use of restriction and modifying enzymes, gel electrophoresis, DNA-fragment isolation, and *Escherichia coli* transformation procedures were carried out according to Maniatis *et al.* (23). DNA sequences at deletion endpoints or of Mu end subclones were determined by the dideoxy sequencing procedure (24) using as template either single-stranded DNA from phage M13mp18 clones (25) or CsCl-purified double-stranded plasmid DNA (26).

Cloning of the Mu Terminal Sequences and Deletion Analysis. The Mu termini were cloned from the pBC177 plasmid, which has the mini-Mu dII4041 (Km^R) element inserted in the pUC9 (Ap^R) plasmid between the *Pst* I and *Hin*dIII sites of the polylinker region with the right terminus oriented proximal to the *Hin*dIII site and the *lac* promoter region (20, 27, 28). The left-end 1006 bp of Mu was cloned by removing the right end of the mini-Mu dII4041 insertion in pBC177 by digestion with *Hin*dIII, ligating, and selecting Ap^R Km^S transformants of strain JM83 (28) to form pAD510. The terminal 203 bp from the left end of Mu was cloned from pAD510 by digesting the 1-kbp *Bam*HI–*Hin*dIII left-end Mu fragment with *Alu* I and ligating with *Bam*HI/*Hin*cII-digested pUC9 vector DNA to form pAD511. The right terminal 117 bp of Mu was cloned by removing the left end of the mini-Mu dII4041 insertion in pBC177 by digestion with *Bam*HI, ligating, and selecting Ap^R Km^S transformants to form pAD500.

Plasmid pAD502, containing a copy of the Mu right end in another location on pUC9, at the *Nde* I site just after the *lac* segment, was made from the pAD500 right-end clone by removing the DNA between the *Bam*HI and *Nde* I sites by digestion with *Bam*HI and *Nde* I, making the ends blunt with mung bean nuclease, ligating, transforming JM83 to Ap^R to form pAD501, and then replacing the *Bam*HI–*Nde* I fragment

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ap, ampicillin; Cb, carbenicillin; Cm, chloramphenicol; Sm, streptomycin; Tc, tetracycline; IHF, integration host factor.

*To whom reprint requests should be addressed.

on the other side of the Mu right end by ligating the smaller *Xmn* I (which cuts in the *bla* gene for Ap^R)–*Hin*dIII fragment of pAD501 to the larger *Xmn* I–*Nde* I fragment of pUC9 after filling in the *Hin*dIII end with Klenow DNA polymerase. A similar scheme was used to construct the pAD503 plasmid with a single copy of the Mu right end at the single *Eco*O109 site of pUC9, which is 195 bp beyond the end of the *lac* segment. A similar plasmid, pAD504, containing two copies of the Mu right end, at the *Pst* I and *Nde* I sites of pUC9, in the same orientation was made by combining the larger *Xmn* I–*Hin*dIII fragment of pAD501 to the smaller *Xmn* I–*Nde* I fragment of pAD500 after filling in the 5′ protruding ends with Klenow polymerase.

Deletions from both sides into the left end of Mu were made, in effect, by subcloning the *Xho* II and *Fnu*DII digestion fragments of the 203-bp *Bam*HI–*Hin*dIII left-end Mu fragment on pAD511 into pUC9. Plasmids pAD512 and pAD513 (harboring deletions Δ512 and Δ513) contain only the terminal 58 and 126 bp of the Mu left end, and pAD514 and pAD515 (harboring deletions Δ514 and Δ515) have Mu left-end sequences from bp 54 to 203 and bp 127 to 203 (see Fig. 2).

Deletions from the inside into the 117-bp right end of Mu were made from the *Bam*HI site (see Fig. 2) of the mini-Mu dII4041 element in pBC4041 (20). The DNA was digested for various times with BAL-31 exonuclease, cleaved at the *Sma* I site located just to the left, ligated with synthetic *Eco*RI linker DNA (GGAATTCC), and used to transform the Mu-lysogenic strain MC1040-2 (20) with selection for Ap^R Km^R. The Mu left end of each deletion plasmid was removed by digesting with *Ssp* I and *Xmn* I, ligating, transforming JM83 to Km^R, and screening for Ap^S.

Deletions originating from outside the Mu right end were constructed, in effect, by subcloning *Fnu*DII and *Alu* I–*Bam*HI fragments of the terminal 117-bp Mu sequences on plasmid pAD500 into pUC9 cleaved with *Hin*cII and *Bam*HI. Plasmids pAD505, pAD506, and pAD507 (harboring deletions Δ505, Δ506, and Δ507) removed 21, 55, and 61 bp from outside the Mu right end (see Fig. 2).

Transduction Assay for Immunity (20). Monomer forms of the Ap^R and Km^R test plasmids were introduced by DNA transformation into the BAC101 and MB1 *recA* strains lysogenic for Mu dII4041 (Km^R) and the Mu dII1678 (Ap^R) mini-Mu and the temperature-sensitive helper phage Mu*cts*. Mu lysates were induced as described (29) and used within a few hours (30). Infection was carried out by mixing 0.5 ml of a fresh overnight culture of the Mu-lysogenic strain M8820-Mu (20) with an equal volume of phage lysate and waiting 30 min at 30°C without shaking for adsorption. The infected cells were diluted 1:10 and grown for 90 min at 30°C to allow expression of the drug-resistance genes before plating with selection for Ap^R and Km^R. Transduction frequencies are the means of three or four experiments.

Conjugation Assay for Immunity. Transposition of a mini-Mu element from a conjugally transmissible F plasmid, pOX38::mini*tet* (Tc^R) (31), to a pUC9-derived test plasmid (Ap^R) with or without Mu terminal sequences was expressed as the frequency of conjugal transfer of the Ap^R marker on the recipient plasmid per transfer of the Tc^R marker on the transmissible plasmid. Mu dII4041 was first introduced onto pOX38::mini*tet* by infection and selection for the linked transfer of Km^R and Tc^R to form pAD540. The donor strains, MG1655.10 containing pAD540 and either pUC9, pAD500, pAD511, or pAD520, were grown overnight at 30°C with appropriate selective drugs. These cultures were diluted 1:100 in LB medium without drugs and an overnight culture of the M8820Mu recipient was diluted 1:50. The cultures were shaken at 30°C for 2 hr, after which time the donors were induced at 42°C for 20 min. Equal volumes of donor and recipient were mixed and incubated at 30°C without shaking for 2 hr. After mating, appropriate dilutions were spread onto plates to select for Sm^R Tc^R and Sm^R Cb^R exconjugants in order to measure the total transfer of pAD540 and transfer of the pAD540-(Ap^R) cointegrates, respectively.

RESULTS

Mu Transposition Immunity. A procedure for selecting mini-Mu insertions in plasmids was used to study the effect of Mu terminal sequences on plasmid targets for Mu transposition (20). Target plasmids derived from pUC9 (Ap^R) were introduced into cells containing mini-Mu dII4041 (Km^R) and a complementing Mu helper prophage in the chromosome, both with the temperature-sensitive Mu repressor mutation *cts*62 (32). These cells were induced for Mu lytic growth and lysates were prepared. Insertions of the mini-Mu into the plasmid were selected as Km^R Ap^R transductants of a Mu-lysogenic cell. For each case, plasmid DNA from several transductants was examined after restriction endonuclease digestion to verify that insertions of the mini-Mu elements had indeed occurred.

Plasmids containing the terminal 203 bp of the Mu left end, pAD511, and the terminal 117 bp of the Mu right end, pAD500, were found to receive mini-Mu insertions 41 and 250 times less frequently, respectively, than did the pUC9 plasmid or the pUC9-derived control plasmid, pAD520, made by inserting the 350-bp *Hin*dIII–*Bam*HI fragment of pBR322 (33) (Table 1).

To show that this phenomenon was not specific to the pUC9 plasmid, a plasmid with a different compatibility group and different drug-resistance genes was used. The Mu right end from plasmid pAD500 was cloned between the *Hin*dIII and *Bam*HI sites of pACYC184 (34). The resulting plasmid, pAD530, received mini-Mu insertions 20 times less frequently than did pACYC184 (Table 1).

Immunity Is Cis-Acting. The effect of an end of Mu on transposition in trans was tested by using a second plasmid, pACYC184 (Cm^R), as a transposition target in cells containing pUC9 and the pUC9-Mu right-end clone, pAD500 (Table 1). In the presence of the pACYC184 plasmid, the pAD500 Mu right-end clone continued to be immune: it received mini-Mu insertions 117 times less frequently than did pUC9. This immunity did not extend in trans to the pACYC184 plasmid, which did not receive mini-Mu insertions less frequently with the Mu right-end clone pAD500 than with pUC9. Actually, pACYC184 received insertions 22 times more frequently with pAD500 than with pUC9. This may

Table 1. Transposition immunity of Mu terminal sequences

Recipient plasmid	Selection*	Transduction frequency†	Relative level
pUC9	Ap	4.0×10^{-6}	(1.0)
pAD511 (pUC9 + Mu L)	Ap	9.8×10^{-8}	41
pAD500 (pUC9 + Mu R)	Ap	1.6×10^{-8}	250
pAD520 (pUC9 + control)	Ap	3.7×10^{-6}	1.1
pACYC184	Cm	5.0×10^{-7}	(1.0)
pAD530 (pACYC184 + Mu R)	Cm	2.5×10^{-8}	20
pUC9 + pACYC184	Ap	1.4×10^{-5}	(1.0)
pAD500 (pUC9 + Mu R) + pACYC184	Ap	1.2×10^{-7}	117
pUC9 + pACYC184	Cm	5.0×10^{-9}	(1.0)
pAD500 (pUC9 + Mu R) + pACYC184	Cm	1.1×10^{-7}	0.05

Mu R, Mu right end; Mu L, Mu left end.
*Selection was for Km^R encoded by the mini-Mu dII4041 element plus Ap^R encoded by pUC9 or Cm^R encoded by pACYC184, as indicated.
†Transduction frequency is expressed as the number of Km^R Ap^R transductants per plaque-forming unit in the lysate.

reflect differences between the pUC9 and pACYC184 plasmids.

Transposition Immunity Can Occur in the Absence of the Mu Late Genes and Mu Lytic Growth. To test for Mu transposition in the absence of Mu lytic growth without the myriad of Mu phage genes, a conjugation assay for transposition was used. Mu dII4041 has only the Mu *c*ts62 repressor and *ner* antirepressor regulatory genes and the *A* and *B* transposition genes. An insertion of mini-Mu dII4041 into the transmissible F factor pOX38::mini*tet* (Tc^R) (31), pAD540, was used as the donor for transposition into pUC9 and the pAD500 and pAD511 clones with the left and right ends of Mu. Selection was for transfer of the Ap^R marker with the F factor. This transfer is dependent on cointegrate transposition to join the nontransmissible pUC9 plasmids to the transmissible F plasmid.

The presence of the Mu left or right end on pUC9 reduced the frequency of mini-Mu dII4041-mediated cointegration by a factor of 78 and 104, respectively (Table 2). The control plasmid pAD520 did not have a reduction in the frequency of insertion. These data indicate that the Mu functions required for the transposition immunity phenomenon include no more than the Mu regulatory and *A* and *B* transposition genes encoded by the mini-Mu element.

Transposition Immunity Is Independent of Position and Increased by Another Copy of a Mu End. The level of immunity was found to be largely independent of the position of the Mu termini within the target plasmid. Approximately the same levels of immunity were seen when the Mu right end was located within the pUC9 polylinker region or at either the single *Nde* I or the single *Eco*O109 site. However, the presence of two copies of the Mu right end (pAD504) increased the immunity level 8–16 times over that already observed for the pUC9 clones with one copy of the right end.

Localization of the Mu Immunity Sequences. The sequences on each end of Mu required for transposition immunity were mapped with deletions extending from each side. Deletions on the left end were made by cloning restriction fragments from the 203-bp Mu left-end fragment on pAD511 (see Fig. 2). Fig. 1*A* (open circles) shows that pAD511 resulted in a factor-of-58 reduction in transduction frequency as compared to pUC9. This decrease is approximately the same as the factor-of-41 reduction seen in the experiments of Table 1. Removal of 77 bp from the inside of the left end, leaving only the terminal 126 bp (Δ513), resulted in the loss of immunity. Removal of an additional 68 bp from the inside, leaving only 58 bp (Δ512), resulted in a return of a small amount of immunity (≈3-fold). For deletions from the outside of the left-end 203-bp segment (Fig. 1*A*, solid circles), approximately the same transduction frequency was seen when 58 bp (Δ514) or 126 bp (Δ515) were removed. Thus, the 77 bp from 127 to 203 from the left side were sufficient for immunity.

Deletions into the right end were made with BAL-31 exonuclease from inside of Mu starting at bp 117 at the *Bam*HI site on mini-Mu dII4041 on plasmid pBC4041 (see Fig. 2). The frequency at which plasmids with partially deleted Mu ends received mini-Mu dII1678 insertions was

Table 2. Conjugation assay of transposition immunity*

Recipient plasmid	Transposition frequency*	Relative level
pUC9	5.2×10^{-3}	(1.0)
pAD500 (pUC9 + Mu L)	6.7×10^{-5}	78
pAD511 (pUC9 + Mu R)	5.0×10^{-5}	104
pAD520 (pUC9 + control)	6.3×10^{-3}	0.8

Mu R, Mu right end; Mu L, Mu left end.

*The transposition of Mu dII4041 from pAD540 to the recipient plasmid was measured as the ratio of the number of conjugated transposition events (selected as Sm^R Cb^R) to the total number of transfers (selected as Sm^R Tc^R).

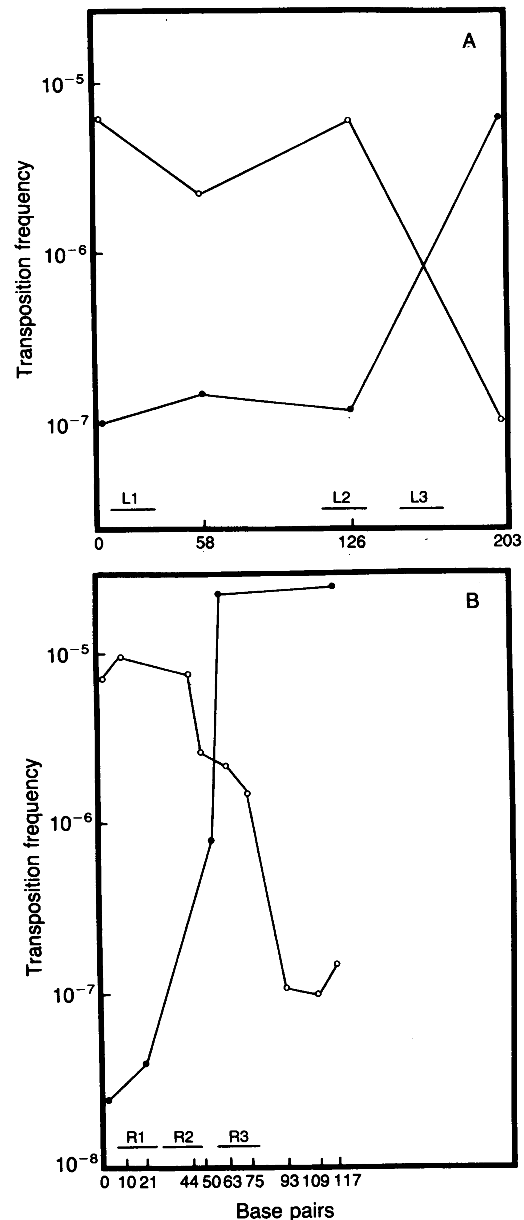

FIG. 1. Localization of Mu cis-acting immunity sequences by deletion analysis of the left and right ends. Transposition frequency is expressed as the number of Ap^R Km^R transductants obtained per plaque-forming unit in the lysate. Deletion endpoints are given in base pairs from either the left (*A*) or right (*B*) end of Mu. ○, Deletions extending from the inside of the Mu ends; ●, deletions extending from the outside of the Mu ends. The positions of the core parts of the six Mu A binding sites (L1–L3 and R1–R3) are indicated.

measured as the frequency of cotransduction of the Km^R plasmid marker and the Ap^R mini-Mu dII1678 phage marker. Transposition immunity increased slightly when the first 8 bp were removed in Δ65 and then decreased nearly logarithmically until the last 44 bp remained with Δ19, which had no significant immunity (Fig. 1*B*, open circles). However, the next deletion, Δ104, which retained the most-terminal 10 bp, actually resulted in an ≈2-fold increase in the transduction frequency as compared to the Δ101 deletion of the complete end.

Deletions into the right terminus from the outside were constructed by subcloning various right-end fragments into pUC9 by using the restriction sites indicated in Fig. 2. The frequency at which these plasmids received mini-Mu dII4041 insertions was measured as the frequency of cotransduction

of the Ap^R plasmid marker and the Km^R Mu dII4041 phage marker. Deletion Δ505, which removed the first 21 bp of the Mu right terminus, affected immunity only slightly (Fig. 1*B*, solid circles). Deletion Δ506, which removed the first 55 bp, had an intermediate level of immunity, whereas deletion of an additional 6 bp (Δ507) almost totally eliminated immunity.

DISCUSSION

The sequences required for transposition immunity were mapped to either Mu end. On the left end the immunity site contained only the innermost of the three binding sites for the Mu A protein (10), and on the right side it contained the innermost two of the three binding sites. These sites do not correspond to the strongest or the weakest binding sites, nor do they correspond to the terminal sequences essential for Mu transposition, which include the outer two binding sites (9, 14). Specifically, on the left side the immunity site was located between bp 127 and bp 203 (Figs. 1*A* and 2), a region that includes the innermost Mu A binding site (L3) and a small part of the next binding site (L2). In contrast, the sequence required for transposition extends from the end to between bp 156 and bp 163, including L1, L2, and part of L3. For partial transposition, at a 15 times lower frequency, the terminal 25 bp with only L1 are sufficient (9). L1 and L3 are both strong binding sites, yet only L3 maps in the immunity region. The terminal 58 bp of Mu, however, containing only the L1 site, did yield a low (2.3) level of immunity, but this same sequence with additional base pairs up to the terminal 126 bp with L1 and part of L2 yielded none.

On the right terminus, the immunity sequences were located between bp 1 and bp 93 with deletions from the inside and between bp 21 and bp 117 with deletions from the outside (Figs. 1*B* and 2). The region in common includes the second (R2) and third (R3) Mu A binding sites. The next deletions from the inside, keeping the terminal 75, 63, or 50 bp but missing part or all of R3, had a reduced level of immunity, as did the next deletion from the outside, retaining bp 56 to bp 117 and missing R2 and the first few nonconserved base pairs of R3. Thus, these deletions implicate two nonoverlapping regions, one containing R2 and the other R3, which each yield partial immunity but which together give full immunity. For transposition, only the terminal 52 bp, containing R1 and R2 but not R3, are sufficient, and the terminal 32 bp are sufficient for transposition at a 30 times lower frequency (9). Only R3 binds Mu A strongly on the right.

As the Mu immunity sites do not correlate with the presence or strength of the A binding sites, they may correlate with another feature or subsequence of the A binding site (14) or with another sequence in this region. An additional sequence in common to the mapped immunity region is a 14-bp stretch of nucleotides located just 5′ of the L3 and R3 Mu A binding sites (Fig. 2) that bears some homology to the integration host factor (IHF) binding consensus sequence (35). These sequences alone, however, do not mediate immunity, since the deletion that removes the outer 61 bp of the Mu right end leaves this sequence and yields a plasmid with no immunity. In addition, a clone of the Mu early operator region known to harbor an IHF binding site, pHK09 (36), did not confer immunity (data not shown). This clone also has the main Mu operators, and its absence of transposition immunity provides further evidence that the Mu c repressor is not involved.

Our results also indicate that no other Mu genes are needed beyond those encoding the A and B proteins and the repressor (*c* gene product) and antirepressor (*ner* gene product) regulatory proteins. These regulatory proteins are probably not involved, since a temperature-sensitive repressor, Mu *cts*62, was used at the high temperature and neither protein was required in the *in vitro* system.

The *in vivo* experiments described here are consistent with the early *in vitro* findings (8, 15) in which mini-Mu transposition preferentially utilized recipient DNA molecules that did not contain mini-Mu insertions. Those *in vitro* experiments did not use isogenic plasmids, as we have used here, that differ only by the addition of short Mu sequences, since it is known that Mu inserts at different frequencies in different plasmids (see Table 1), as well as in different regions of the chromosome (37) and within particular genes (37–40).

Recent *in vitro* experiments (16) have shown that the Mu B protein interacts with target DNA to facilitate its capture by a donor complex. Immunity could occur when the B protein that becomes associated with an immune target molecule interacts with a transposition complex bound at an immunity site and is either removed, taken up into the transposition complex with the A protein, or otherwise made inactive or unavailable for transposition. However, the same B protein, when interacting with a Mu donating protein complex from another molecule, must function for a productive transposition event.

Earlier experiments have addressed the distribution of multiple Mu insertions without yielding evidence for transposition immunity. Studies with the electron microscope

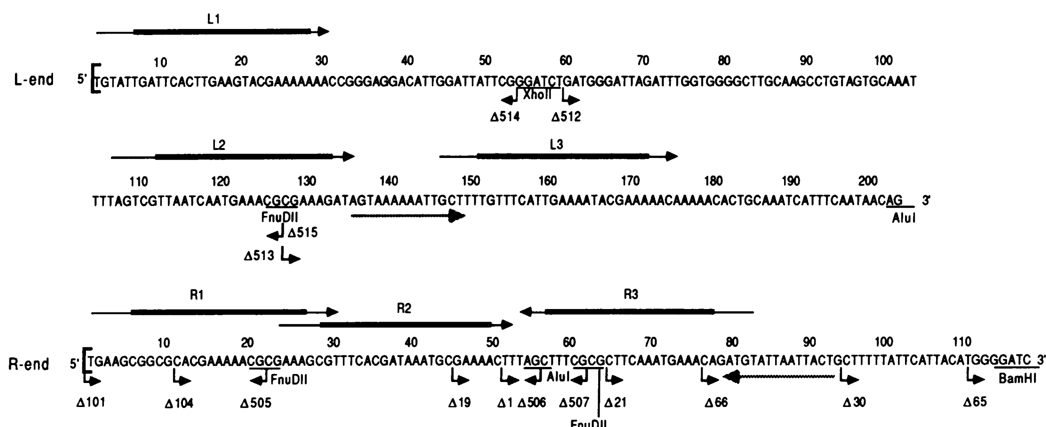

FIG. 2. Nucleotide sequences involved in transposition immunity at the left (L) and right (R) ends of bacteriophage Mu. Numbers above the sequences denote the distance, in base pairs, from each end. The arrows above the sequences indicate the regions protected from DNase by Mu A protein binding (10), with the thicker black lines marking the 22-bp consensus sequence (9, 14). Arrows below the sequence indicate the nucleotides removed by the deletions used to localize the immunity sequence. The stippled arrow beneath the Mu sequences at each end denotes nucleotides that bear homology to the IHF binding consensus (35).

indicated that Mu insertions were clustered (41), which would not be predicted from a regional immunity model. A possible explanation for this comes from the timing of the transpositions. If it takes time for immunity to be established, then insertions that have just been made may not yet have immunity established, and additional insertions may in fact tend to occur nearby if the transposition machinery is physically still located in the target region. A similar phenomenon has been seen for the Tn*3* element, which also has immunity: insertions into a plasmid are frequently accompanied by a second insertion that apparently is made at nearly the same time, whereas insertions into a plasmid that already has an established insertion occur at a much lower frequency (42). An alternative explanation for the electron microscope observations is that large chromosomal fragments may be examined less frequently than the shorter ones, so that closer inserts would be more likely to be seen.

In another experiment, insertions into a large F′ *lac–proAB* transmissible plasmid were not less frequent when it already contained a Mu insertion (R. Harshey, personal communication). Possible explanations for the failure to see immunity with the F′ *lac–pro* plasmid are that the large size of this plasmid (90 kbp) may have masked the immunity effect, it may already have sequences that resemble the Mu termini and confer immunity, or it may have some sort of immunity-blocking sequence or segregated DNA domains.

The phenomenon of Mu transposition immunity may provide an explanation for how Mu avoids inserting into itself. This immunity, however, must be limited to regions near Mu, since it must repeatedly transpose during lytic growth. Consistent with a regional immunity model are observations with mini-Mu elements containing plasmid replicons designed to clone DNA. Clones using these elements are made from insertions on each side of a gene (29, 43). When mini-Mu insertions are located near a particular gene prior to induction of transposition in the *in vivo* cloning process, clones of that gene are found 50 times less frequently than of other chromosomal genes located far from the established mini-Mu insertion (E. Groisman and M.J.C., unpublished results). This observation is also consistent with the model that transposition immunity is not fully established immediately after each round of transposition in a cell undergoing Mu lytic growth.

The transposition immunity observed for Mu in this study is similar to that which has been observed for the Tn*3* class of transposons (44–46). These are thought to be distantly related to Mu in that they also duplicate 5 bp upon insertion. The center of the single Tn*3* transposase binding site at each end has the sequence (RCGAAAR, where R = A or G) found in each of the six Mu A transposase binding sites (8, 47). Tn*3* immunity requires either of these transposase binding sites at each end starting between 8 and 17 bp from the outside and extending to between 35 and 38 bp (J. Kans and M.J.C., unpublished results). Although Tn*3* does not have the equivalent of the Mu B gene, it is interesting that the Tn*3* *tnpA*-encoded transposase is large enough to be equivalent to a fusion of the Mu A and B proteins, so that the Tn*3* termini may include binding sites for two domains of Tn*3* corresponding to the Mu A and B equivalent parts (48).

Another interesting feature of the Mu terminal deletions used here is that the deletion that leaves the outer 10 bp of the Mu right end resulted in a reproducible 2-fold increase in the frequency at which a mini-Mu inserted into the plasmid. A similar phenomenon was seen for Tn*3* with deletions leaving 28 and 29 bp from the end (ref. 46; J. Kans and M.J.C., unpublished results). This "negative transposition immunity" may be due to the retention of a partial transposase binding site without an immunity sequence.

We thank P. Higgins for the Mu immunity-region clone. A.D. was supported by National Institutes of Health Postdoctoral Fellowship GM11187 and N.E.K. was supported in part by Traineeship GM07543-08. This work was supported by Grant GM29067 from the National Institutes of Health.

1. Toussaint, A. & Resibois, A. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. (Academic, New York), pp. 105–158.
2. Chaconas, G. (1987) in *Phage Mu*, eds. Symonds, N., Toussaint, A., van de Putte, P. & Howe, M. M. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 137–157.
3. Howe, M. M. & Bade, E. G. (1975) *Science* **190**, 624–632.
4. Bukhari, A. I. (1976) *Annu. Rev. Genet.* **10**, 389–411.
5. O'Day, K. J., Schultz, D. W. & Howe, M. M. (1978) in *Microbiology 1978*, ed. Schlessinger, D. (Am. Soc. Microbiol., Washington, DC), pp. 48–51.
6. Faelen, M., Huisman, O. & Toussaint, A. (1978) *Nature (London)* **271**, 580–582.
7. Waggoner, B. T., Pato, M. L., Toussaint, A. & Faelen, M. (1981) *Virology* **113**, 379–387.
8. Mizuuchi, K. (1983) *Cell* **35**, 785–794.
9. Groenen, M. A. M., Timmers, E. & van de Putte, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2087–2091.
10. Craigie, R., Mizuuchi, M. & Mizuuchi, K. (1984) *Cell* **39**, 387–394.
11. Schumm, J. W. & Howe, M. M. (1981) *Virology* **114**, 429–450.
12. Chaconas, G., Harshey, R. M., Sravetnick, J. & Bukhari, A. I. (1981) *J. Mol. Biol.* **150**, 341–359.
13. Faelen, M., Toussaint, A. & Resibois, A. (1979) *Mol. Gen. Genet.* **176**, 191–197.
14. Groenen, M. A. M. & van de Putte, P. (1986) *J. Mol. Biol.* **189**, 597–602.
15. Craigie, R. & Mizuuchi, K. (1987) *Cell* **51**, 493–501.
16. Adzuma, K. & Mizuuchi, K. (1988) *Cell* **53**, 257–266.
17. Mogutov, M. A., Velikodvorskaya, G. A., Kobets, N. S. & Piruzyan, E. S. (1985) *Genetica* **21**, 927–935.
18. Reyes, O., Beyou, A., Mignotte-Vieux, C. & Richaud, F. (1987) *Plasmid* **18**, 183–192.
19. Guyer, M. S., Reed, R. R., Steitz, J. A. & Low, K. B. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 135–140.
20. Castilho, B. A., Olfson, P. & Casadaban, M. J. (1984) *J. Bacteriol.* **158**, 488–495.
21. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
22. Kupersztoch, Y. & Helinski, D. (1973) *Biochem. Biophys. Res. Commun.* **54**, 1451–1459.
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
24. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
25. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26**, 101–106.
26. Chen, E. Y. & Seeburg, P. H. (1985) *DNA* **4**, 165–170.
27. Castilho, B. A. (1984) Ph.D. Dissertation (Univ. of Chicago, Chicago, IL).
28. Vieira, J. & Messing, J. (1982) *Gene* **19**, 259–268.
29. Groisman, E. & Casadaban, M. J. (1986) *J. Bacteriol.* **168**, 357–364.
30. Bukhari, A. I. & Ljundquist, E. (1977) in *DNA Insertion Elements, Plasmids and Episomes*, eds. Bukhari, A. I., Shapiro, J. A. & Adhya, S. L. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 749–756.
31. Way, J. C., Davis, M. A., Morisato, D., Roberts, D. E. & Kleckner, N. (1984) *Gene* **32**, 369–379.
32. Howe, M. (1972) Ph.D. Thesis (Massachusetts Institute of Technology, Cambridge, MA).
33. Sutcliffe, J. G. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 77–90.
34. Chang, A. C. Y. & Cohen, S. N. (1978) *J. Bacteriol.* **134**, 1141–1156.
35. Craig, N. & Nash, H. (1984) *Cell* **39**, 707–716.
36. Krause, H. M. & Higgins, P. (1986) *J. Biol. Chem.* **261**, 3744–3752.
37. Bukhari, A. I. & Zipser, D. (1972) *Nature (London)* **236**, 240–243.
38. Daniell, E., Roberts, R. & Abelson, J. (1972) *J. Mol. Biol.* **69**, 1–8.
39. Raibaud, O. R. M., Braun-Breton, C. & Schwartz, M. (1979) *Mol. Gen. Genet.* **174**, 241–248.
40. Silhavy, J., Brickman, E., Bassford, P., Jr., Casadaban, M. J., Shuman, H. A., Schwartz, V., Guarente, L., Schwartz, M. & Beckwith, J. R. (1979) *Mol. Gen. Genet.* **174**, 249–259.
41. Harshey, R. M., McKay, R. & Bukhari, A. I. (1982) *Cell* **29**, 561–571.
42. Tu, D. & Cohen, S. N. (1980) *Cell* **19**, 151–160.
43. Groisman, E. A., Castilho, B. A. & Casadaban, M. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1480–1483.
44. Heffron, F. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. (Academic, New York), pp. 223–260.
45. Robinson, M. K., Bennett, P. M. & Richmond, M. H. (1977) *J. Bacteriol.* **129**, 407–414.
46. Lee, C.-H., Bhagwat, A. & Heffron, F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6765–6769.
47. New, J. H., Eggleston, A. K. & Fennewald, M. (1988) *J. Mol. Biol.* **201**, 589–599.
48. Kamp, D. (1987) in *Phage Mu*, eds. Symonds, N., Toussaint, A., van de Putte, P. & Howe, M. M. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 259–269.