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Lethal Transposition of Mu*d* Phages in Rec⁻ Strains of *Salmonella typhimurium*

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ABSTRACT

Under several circumstances, the frequency with which Mu*d* prophages form lysogens is apparently reduced in *rec* strains of *Salmonella typhimurium.* Lysogen formation by a Mu*d*I genome (37 kb) injected by a Mu virion is unaffected by a host *rec* mutation. However when the same Mu*d*I phage is injected by a phage P22 virion, lysogeny is reduced in a *recA* or *recB* mutant host. A host *rec* mutation reduces the lysogenization of mini-Mu phages injected by either Mu or P22 virions. When lysogen frequency is reduced by a host *rec* mutation, the surviving lysogens show an increased probability of carrying a deletion adjacent to the Mu*d* insertion site. We propose that the *rec* effects seen are due to a failure of conservative Mu transposition. Replicative Mu*d* transposition from a linear fragment causes a break in the host chromosome with a Mu prophage at both broken ends. These breaks are lethal unless repaired; repair can be achieved by Rec functions acting on the repeated Mu sequences or by secondary transposition events. In a normal Mu infection, the initial transposition from the injected fragment is conservative and does not break the chromosome. To account for the conditions under which *rec* effects are seen, we propose that conservative transposition of Mu depends on a protein that must be injected with the DNA. This protein can be injected by Mu but not by P22 virions. Injection or function of the protein may depend on its association with a particular Mu DNA sequence that is present and properly positioned in Mu capsids containing full-sized Mu or Mu*d*I genomes; this sequence may be lacking or abnormally positioned in the mini-Mu*d* phages tested.

TRANSPOSITION is an integral part of the life cycle of bacteriophage Mu (TAYLOR 1963; BUKHARI 1976; TOUSSAINT and RESIBOIS 1983). Upon infection, the Mu genome transposes from the infecting DNA fragment and integrates into the host chromosome. This initial transposition event is conservative; there is minimal replication of Mu sequences and the Mu sequences are released from their association with flanking donor sequences (reviewed by HARSHEY 1987). After integration into the chromosome, the Mu genome transposes replicatively to new chromosomal sites (reviewed by PATO and WAGGONER 1987). During the period of replicative transposition, the bacterial chromosome is subjected to multiple rearrangements including deletions, duplications and inversions (TOUSSAINT and RESIBOIS 1983). A general model for the process of replicative transposition has been proposed by SHAPIRO (1979) and in vitro experiments have demonstrated and elaborated upon this model (CRAIGIE and MIZUUCHI 1985; for reviews see MIZUUCHI and CRAIGIE 1986; MIZUUCHI and HIGGINS 1987).

The detailed mechanism of the initial conservative transposition event remains unclear. Although normal levels of both replicative and conservative transposition require both the Mu *A* and *B* functions, both functions can occur at a reduced level with only the Mu *A* function (CHACONAS *et al.* 1985). The two types of transposition events may occur by substantially different mechanisms (CHACONAS 1987) or may be variations of a single process (CRAIGIE and MIZUUCHI 1985). It has been proposed that conservative transposition requires participation of a tail protein of phage Mu, which is injected with the DNA upon phage infection (GLOOR and CHACONAS 1986). We believe that the data presented here can best be explained in terms of this injected protein.

Both conservative and replicative modes of Mu transposition are thought to be independent of the host recombination system (*recA.B.C*). Therefore it was surprising to observe that Mu*d*I, a transposon derived from phage Mu, shows an apparently decreased ability to transpose from a P22-transduced fragment into the chromosome when the recipient carries a *rec* mutation (HUGHES, OLIVERA and ROTH 1987). Since this effect of Rec deficiency on lysogen recovery was not observed when the Mu*d*I genome was injected by a Mu virion, it appeared that some aspect of virion structure might contribute to the process of Mu transposition or lysogenization.

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TABLE 1

Strain list

Strain[a]	Genotype
Source: Laboratory collection	
LT2	Wild type
TT3758	*hisG46 recB503*::Tn*10*
TT7216	*his-9944*::MudI
TT8050	*ilvA99 pyrF231 malA110 gal-851 metA27 trpE2 hisF1009 rspL201 xyl-1 musA1* Mu*cts*62 MudI
TT8353	pLP103-63-3 (MuA^+B^+)
TT8785	*nadA219*::MudA
TT10199	*nadA56*/F′152-2
TT10288	*hisD9953*::MudJ *his-9941*::MudI
TT10377	LT2/pLP103-32-2 (Mu A^+)
TT10838	*recA1*
TT11183	*srl-203*::Tn*10d*-Cam
TT10502	*musA1*
TT10503	*musA1 recA1*
TT10504	*musA1 recB503*::Tn*10*
TT10505	*musA1 recA1 recB503*::Tn*10*
TT12116	*nadA213*::Tn*10*/F′152-2 nad^+ zzf-1873::Mu*d*F
Source: This work	
TT14547	*purF2054*::MudJ *musA1 zxx-3675*::Mu*p*Ap1
TT14548	*musA1 zxx-3675*::Mu*p*Ap1
TT14549	*musA1*/pLP103-32-2 (Mu A^+)
TT14550	*musA1 recA1*/pLP103-32-2 (Mu A^+)
TT14555	*recA1*
TT14556	*recB503*::Tn*10*
TT14558	*recA1 recB503*::Tn*10*
TT15216	*recA1*/pLP103-32-2 (Mu A^+)
TT15220	*recB503*::Tn*10*/pLP103-32-2 (Mu A^+)
TT15221	*recA1 recB503*::Tn*10*/pLP103-32-2 (Mu A^+)
TT15881	*ara-663*::Mu*d*II1681
TT17337	*musA1 zxx-3675*::Mu*p*Ap1 *ara-662*::Mu*d*II1681

[a] All strains are derivatives of *S. typhimurium* LT2.

We have pursued these observations and suggest here that the reduced recovery of Mu*d* lysogens following P22 transduction of Mu-derived elements into Rec⁻ hosts is due to increased host killing rather than a failure of transposition. We propose that lethality is due to unrepaired breaks in the host chromosome which occur when the phage genome transposes replicatively (instead of conservatively) from the linear injected fragment. Results are interpreted to suggest that conservative transposition requires a protein which is normally injected with the Mu genome; injection of this protein requires a Mu virion. The injection or function of the protein may require association with a particular Mu DNA sequence that is missing or improperly positioned in the smaller mini-Mu elements.

MATERIALS AND METHODS

Bacteria: All strains used are derived from *Salmonella typhimurium* LT2. A list of strains is shown in Table 1.

Media: Minimal medium was either the E medium of VOGEL and BONNER (1956) or NCE medium (BERKOWITZ *et al.* 1968). The E medium was supplemented with glucose. The NCE medium was supplemented with D-galactose. The carbon source was added to a concentration of 0.2%. The complex medium was nutrient broth (0.8%; Difco) with added sodium chloride (0.5%). MacConkey-indicator plates (Difco) were supplemented with 1% D-galactose. Auxotrophic supplements were added to minimal media at concentrations described by DAVIS, ROTH and BOTSTEIN (1980). Unless otherwise indicated antibiotics were used at the following concentrations in minimal and complex media, respectively: kanamycin: 125 μg/ml and 50 μg/ml; ampicillin: 15 μg/ml and 30 μg/ml. D-galactose and all antibiotics were obtained from Sigma Chemical Company.

Transductional methods: The high frequency generalized transducing mutant of bacteriophage P22 HT105/1 *int-201* (SCHMIEGER 1972) was used for all transductional crosses. P22 transducing lysates were grown on Gal⁻ P22-resistant strains by introducing an *E. coli* F′ Gal⁺ plasmid (F′152-2) which provides the missing *gal* functions. In transduction crosses involving selection for drug resistance, equal volumes of saturated cultures of recipient cells and donor lysates were mixed and preincubated in liquid medium at 37° before spreading onto selective medium. The preincubation period was 20 min when ampicillin resistance was selected and 45 min when kanamycin resistance was selected. Transductants were first purified selectively and phage-free colonies were identified as light colonies formed following streaking on green indicator plates (CHAN *et al.* 1972). Phage sensitivity of transductants was confirmed by cross streaking with P22 H5 (a clear plaque mutant of phage P22). Transduction crosses using Mu*cts*62 and Mu*p*Ap1 were done according to BERMAN, ENQUIST and SILHAVY (1981).

Identification and analysis of *nadA-gal* deletions: Gal⁻ strains were tested for Nad⁻ and Bio⁻ phenotypes on minimal plates to which nicotine acid or biotin or both were added. The *aroG* phenotype of the *nadA-gal* deletion mutants was determined according to ALPER and AMES (1975). All *nadA-gal* deletion strains isolated are *aroG*⁻ and the majority are phenotypically Dhb⁻ (deficient in synthesis of dihydroxybenzoate). Growth of Dhb⁻ strains is inhibited by citrate, because these strains are deficient in transport of iron (ALPER and AMES 1975). All Dhb⁻ deletions are also Bio⁻.

Determination of the viability of *rec* strains: The optical density of overnight cultures of *rec*⁺, *recA*, *recB*, or *recA recB* strains of *S. typhimurium* was measured using a Klett meter. The cultures were diluted and plated onto complex medium and the number of viable cells/ml/Klett unit of the culture was estimated. The viability of the *rec*⁺ strain was defined as 1. The viability of the *recA* strain was generally 0.66; the *recB* strain was 0.29 and the *recA recB* double mutant was 0.2

RESULTS

Transduced mini-Mu elements require host *rec* function for lysogeny: Previously reported effects of host *rec* mutations on Mu*d* phage inheritance involved experiments with the Mu*d*I(Ap Lac) element (38 kb) derived from phage Mu by CASADABAN and COHEN (1979). It was found that host *rec* mutations reduce the recovery of lysogens formed by Mu*d*I elements injected by P22; no such Rec-effect was seen for the same Mud element injected by a Mu virion (HUGHES, OLIVERA, and ROTH 1987).

To determine whether smaller derivatives of phage Mu are also subject to this Rec-effect, we tested a

FIGURE 1.—The genetic maps of Mu and the Mu*d* phages that were used in this work; all of these phages contain the *cts*62 mutation. The map of Mu was adapted from HOWE (1987). The maps of Mup*Ap*1, Mu*d*I, Mu*d*II1681 and Mu*d*J were adapted from FAELEN (1987). The map of Mu*d*F was deduced from the maps of Mu*d*J and a mini-Mu *lac* transposon that was constructed by CHACONAS *et al.* (1981). The filled in boxes represent nontransposable Tn*3* sequences. In Mup*Ap*I and Mu*d*I these sequences encode Ap^R. The Mu*d*F genome contains a truncated portion of the Ap^R determinant of Tn*3* and does not encode Ap^R. The boxes with horizontal stripes represent nontransposable Tn*5* sequences. The mottled boxes represent *E. coli lac* operon sequences. Mu*d*F encodes the entire *lac* operon of *E. coli*; the *lac* sequences present in Mu*d*I and Mu*d*J do not include the *lac* promotor but can generate *lac* operon fusions to promoters located near the insertion side. The open boxes represent *E. coli trp* sequences. The box with vertical stripes represents an IS121 insertion in Mu*d*I.

mini-Mu*d* element constructed by CHACONAS *et al.* (1981) and modified by R. V. SONTI and J. R. ROTH (unpublished results). This small (14 kb) transposition-defective element, referred to as Mu*d*F, carries a complete *lac* operon, a kanamycin resistance determinant (Km^R), about 1 kb of Mu sequences, including the *c* gene (at the left end) and about 1.5 kb at the right (S) end; it does not include the Mu *A* and *B* transposition functions. The genomes of Mu*d*F and other Mu derivatives used here are diagrammed in Figure 1.

To test the effect of host *rec* mutations on Mu*d*F transposition, we performed P22-mediated transduction crosses. The donor strain (TT12116) carries Mu*d*F inserted in an *E. coli* F′ plasmid carried by a *S. typhimurium* strain; the recipient is a Salmonella strain lacking the F′ plasmid. The *Escherichia coli* sequences flanking the donor Mu*d*F insertion site are sufficiently different from Salmonella chromosomal sequences to prevent inheritance of Mu*d*F by homologous recombination. To permit transposition of the transduced Mu*d*F element, the recipient strain carries a plasmid with the Mu*A* transposase gene expressed at a constitutive level (VAN LEERDAM, KARREMAN and VAN DE PUTTE 1982; M. HOWE, personal communication). The Mu *ner* gene is not included. This plasmid provides a low level of transposition proficiency. Selection was made for Lac$^+$ transductants. Since the Mu*d*F element includes an entire *lac* operon (with promoter), all transductants which inherit Mu*d*F will show a Lac$^+$ phenotype. Because recombinational inheritance is prevented by lack of sequence homology, Mu*d*F is inherited only by transposition from the transduced fragment. This is demonstrated by the absence of Lac$^+$ transductants in *rec*$^+$ host strains lacking the Mu*A* plasmid.

Transpositional inheritance of the mini-Mu, Mu*d*F is greatly reduced in *recA*, *recB* and *recA recB* recipient

TABLE 2

Effects of *rec* mutations on recovered lysogens of mini-Mu phages injected by P22 virions

Recipient strain no.	Genotype of recipient	Transposition functions in recipient[a]	No. of Mu*d*F lysogens (lac⁺ transductants)	Relative frequency of lysogens
LT2	*rec*⁺	None	0	0
TT10377	*rec*⁺	Mu*A*	3132	1
TT15216	*recA*	Mu*A*	482	0.15
TT15220	*recB*	Mu*A*	731	0.23
TT15221	*recA* *recB*	Mu*A*	0	0

Recipient strains were grown overnight in complex medium containing ampicillin (50 μg/ml). A single P22 transducing lysate grown on strain TT12116 (carrying a MudF lysogen in an *E. coli* F plasmid) was used to infect recipient cells at a multiplicity of 2. Cells were plated to select Lac⁺ transductants at 37°. The number of transductants was corrected for a recipient viability factor calculated as described in MATERIALS AND METHODS.

[a] Mu*A* plasmid was obtained from P. VAN DE PUTTE and is described in VAN LEERDAM, KARREMAN and VAN DE PUTTE (1982).

strains (see Table 2). Either a *recA* or a *recB* mutation reduce the frequency of recovered lysogens by about 5-fold (after adjusting for the reduced frequency of viable cells in *rec* mutant strains). A *recA recB* double mutant recipient strain shows a greater reduction in Mu*d*F inheritance. Results similar to these have also been obtained with the mini-Mu elements, Mu*d*J and Mu*d*I1681, diagrammed in Figure 1.

Transposition-proficient mini-Mu genomes show *rec* effects on lysogeny: The Mu*d*I1681 mini-Mu element (12 kb) encodes both Mu *A* and *B* functions (CASTILHO, OLFSON and CASADABAN 1984). Transposition of this element does not require a plasmid to supply transposition functions. Inheritance of this mini-Mu is reduced in Rec⁻ strains (see Table 3), indicating that Rec⁻ effects on mini-Mu transposition from P22-transduced fragments are seen even when the Mu derivative produces its own regulated transposase; therefore the effect does not depend on provision of a constitutive, plasmid-borne transposase.

Mini-Mu phages show a Rec⁻ effect even when injected by Mu virions: To test the behavior of mini-Mu phages packaged in Mu virions, a double lysogen was constructed (TT14547) that carries the mini-Mu element Mu*d*J(Km, Lac) inserted in the *purF* gene of *S. typhimurium* and a plaque-forming Mu derivative at an unidentified site in the chromosome. This mini-Mu*d*J element is the Mu*d*I1734 element constructed by CASTILHO, OLFSON and CASADABAN (1984). The plaque-forming Mu derivative used here is phage Mu*p*Ap1, constructed by LEACH and SYMONDS (1979). When a double lysogen of these two strains is induced by shifting to high temperature (see MATERIALS AND METHODS), a mixed lysate is produced that includes Mu virions that have packaged either the Mu*p*Ap1 or Mu*d*J genomes. By using this lysate as donor in a transduction cross selecting for inheritance of Km^R, we can follow the integration of the Mu*d*J element; Ap^R transductants have acquired the Mu*p*Ap1 phage.

In Table 4, it can be seen that the number of Km^R transductants is reduced 20-fold in the *recA* mutant recipient. None of the Km^R transductants occurred by homologous recombination, since no Km^R transductants were found in *rec*⁺ recipients that lacked the Mu *A* producing plasmids. Furthermore none of the Km^R transductants show the purine requirement characteristic of the donor Mu*d*J insertion, supporting the conclusion that all inheritance occurs by transposition. Apparently the homologous sequences flanking the insertion are insufficient to support homologous recombination at a frequency detectable in this experiment. At the multiplicity of infection used in Table 4, one might have expected the coinfecting Mu*p*Ap1 phages to provide transposase and allow some transposition without the Mu *A* plasmid. Since no Km^R transductants were seen in the recipient lacking the Mu *A* plasmid, it appears that the preferential *cis* action of Mu transposase prevents *trans* activation of Mu*d*J under these conditions. At a higher helper multiplicity, Mu*p*Ap1 does provide transposase for Mu*d*J (see below).

The Rec effects described above are not due to effects of *rec* mutations on the expression or copy number of the recipient Mu A plasmid providing transposase function. The same cross was performed with no Mu *A* plasmid in the recipient; transposition functions were provided by addition of extra transposition-proficient Mu*p*Ap1 helper phage. Therefore both Mu *A* and *B* functions were supplied only by Mu-derived genomes able to provide normal regulation. Results in Table 5 demonstrate that Km^R transductants are seen and their frequency is reduced in a *recA* recipient strain. Thus the presence of a host *recA* mutation reduces the frequency of Mu*d*J lysogens regardless of whether transposition functions are supplied by a plasmid or by a helper Mu phage. As expected from the previous results of HUGHES, OLIVERA and ROTH (1987), inheritance of the Mu*p*Ap1 helper phage itself is not subject to the effect of *recA* (see Table 6). Thus the large Mu genomes are themselves protected from *rec* effects when injected by a Mu virion, but they do not provide this protection *in trans* to the coinjected Mu*d*J genomes, even when they provide the transposition function.

Increasing transposition function reduces the inheritance of transduced Mu*d*F element: The behavior of injected Mu*d*F elements in recipients expressing both the Mu *A* and *B* genes suggested that lethal transposition might reduce the yield of lysogens in Rec⁻ strains. The Mu *A* function alone is sufficient for transposition, but the frequency of Mu transposition is enhanced 50-100 fold if the accessory Mu*B*

TABLE 3

Effect of *rec* mutations on transposition of a transposition-proficient mini-Mu prophage injected by P22 virions

Recipient strain no.	Recipient genotype	Total Km^R transductants	No. of transpositions (Ara$^+$, Km^R)	Corrected no. of transpositions[a]	Relative frequency of transposition
LT2	*rec*$^+$	11172	10278	10278	1
TT14555	*recA*	535	535	1009	0.098
TT14556	*recB*	1	1	11	0.001
TT14558	*recA, recB*	0	0	0	0

Recipient strains were grown overnight in complex medium at 30°. A single transducing lysate (P22 int, HT) was grown on donor strain TT15884 (*ara-663*::Mu*d*II1681) and used to infect all recipients strains at the same multiplicity (<2) based on culture turbidity. Transductants were selected on nutrient broth with kanamycin at 30° and transductants were replica-plated to MacConkey-arabinose-kanamycin medium to identify lysogens that had inherited Km^R by transposition (Ara$^+$) or by homologous recombination (Ara$^+$).

[a] The number of transposition transductants was corrected for recipient cell viability as described in MATERIALS AND METHODS (viability of *recA* cells was 0.53, of *recB* cells was 0.087 and of *recA, recB* cells was 0.033).

TABLE 4

Effect of *rec* mutations on transposition of mini-Mu prophages injected by Mu virions

Recipient strain no.	Genotype of recipient	Transposition functions provided by recipient	No. of Mu*d*J lysogens (Km^r transductants)	Relative frequency of lysogens
TT10502	*rec*$^+$	None	0	0
TT10503	*recA*	None	0	0
TT14549	*rec*$^+$	Mu *A*	1971	1
TT14550	*recA*	Mu *A*	103	0.05

Multiplicity of infection was 0.6–0.75. The donor lysate in all crosses was made by inducing strain TT14547 [*purF*::Mu*d*J(Km), Mu*p*(*Ap*)]. Lysates were prepared, titred and transduction crosses were performed as described in BERMAN, ENQUIST and SILHAVY (1981). All recipients carry a *mus* mutation to permit infection by Mu particles. Transductions were done at 30°. The numbers of recombinants were corrected for the reduced viability of the *recA* strain as described in MATERIALS AND METHODS.

function is also provided (FAELEN, HUISMAN and TOUSSAINT 1978; O'DAY, SHULTZ and HOWE 1978; Chaconas *et al.* 1984). The effect of this accessory function on transduced mini-Mu*d* (Mu*d*F) genomes was seen in a P22-mediated transduction cross. In this case, the Mu*d*F prophage in strain TT12116 was transduced into two isogenic recipients, one expressing only the Mu *A* gene, the other expressing both Mu *A* and Mu *B* genes constitutively from a plasmid; selection was for inheritance of the Km^R determinant associated with Mu*d*F. In these crosses, provision of the accessory Mu *B* function caused a strong *reduction* in recovery of Mu*d*F lysogens (Table 7). This is expected if, under these conditions, the higher level of transposition functions permit multiple rounds of replicative transposition, which is frequently lethal to the host cell. This result, while not surprising, suggested the possibility that the reduced frequency of Km^R Mu*d* lysogens in *rec* strains (described above) might also have been due to the lethal effects of replicative transposition rather than reduced ability to transpose.

Explanations of the *rec* effects: The effects of recipient *rec* mutations, noted above, could be due to a variety of causes. (1) The *rec* mutations might increase copy number or expression of the plasmid-born Mu *A* gene, leading to increased transposition and excessive killing. Alternatively *rec* alleles might reduce transposase expression to a level that limits the likelihood of the initial transposition. We think both these possibilities are rendered unlikely, since the effect of *rec* mutations is also seen when transposase is provided by Mu derivatives that regulate transposase genes in a normal way. (2) The *rec* alleles might alter host sensitivity to killing by the P22 transducing phage or the several sorts of Mu phages provided. We think this is unlikely because the same *rec* alleles do not impair inheritance of plasmids by P22 transduction or inheritance of Tn*10*, Tn*10*dTet or Tn*5* by transposition from transduced fragments (R. V. SONTI and D. H. KEATING, unpublished). Similarly, these *rec* alleles do not affect lysogen formation by full sized Mu derivatives with normally regulated transposition functions when their genome is injected by a Mu capsid. Thus the observation of a *rec* effect seems to depend on the nature of the injecting virion and the size of genome injected, but not on the source of the transposase function.

These various possibilities can also be controlled by producing both a full-sized Mud derivative and a mini-Mud in a single donor strain and using the mixed lysate to infect a single host culture, observing the effects of *rec* alleles in the recipient on inheritance of the two genomes (by lysogen formation). These results are presented in Table 8. A lysate was prepared on a strain (TT17337) that carries two transposition-proficient Mu*d* derivatives, the mini-Mu Mu*d*II1681(Km) and the full-sized, plaque-forming phage Mu*p*Ap1. This lysate contains both types of genomes in Mu capsids. As seen in Table 8, a cell culture infected by this lysate yields both Km^R lysogens (of the mini-Mu*d*) and Ap^R lysogens (of the full-sized) Mu at a ratio of about 1:4.6. When the same lysate infects a *recA* host

TABLE 5

Effect of a *recA* mutation on the transposition of mini-Mud phages in Mu virions during mixed infection with helper *Mud* phages

Recipient strain no.	Genotype of recipient	Extra helper Mu*d* phage	Multiplicity of infection (including helper phage)	No. of Mu*d*J lysogens (Km^r transductants)	Relative frequency of lysogens
TT10502	*rec*$^+$	Absent	0.6	0	0
TT10503	*recA*	Absent	0.6	0	0
TT10502	*rec*$^+$	Present	2.1	1498	1
TT10503	*recA*	Present	2.1	191	0.13

A plaque-forming Mu*d* phage called Mu*p*Ap1 was used as the helper phage. Mu*p*Ap1 was obtained from M. M. HOWE. The source of Mu*d*J sequences was a lysate on TT14547, which carries both a Mu*d*J(Km) and a Mu*p*Ap1 lysogen. Additional helper phage functions in the transduction cross were provided by Mu particles that were obtained by inducing the Mu*p*Ap1 lysogen (TT14548). Mu lysates were grown, titred and transductions were done as described in BERMAN, ENQUIST and SILHAVY (1981). All recipients carry a *mus* mutation to permit infection by Mu particles. For transduction crosses in which extra helper phage were provided, roughly two thirds of the helper Mu particles (1.5/cell) were from the exogenous lysate and one third (0.6/cell) from the mixed lysate. The transductions were done at 30°. The reduced viability of the *recA* strain was taken into account as described in MATERIALS AND METHODS.

TABLE 6

Effect of a *recA* mutation on transposition of Mu*p*Ap1 phages infected by a Mu virions

Recipient strain no.	Genotype of recipient	Multiplicity of infection	No. of Mu*p*Ap1 lysogens (Ap^r transductants)	Relative frequency of lysogens
TT10502	*rec*$^+$	10^{-5}	4273	1
TT10503	*recA*	10^{-5}	3244	0.76
TT10502	*rec*$^+$	10^{-7}	82	1
TT10503	*recA*	10^{-7}	39	0.48

The donor strain was TT14547. Mu*p*Ap1 lysates were prepared, titred and transduction crosses were performed as described in BERMAN, ENQUIST and SILHAVY (1981). The transductions were done at 30°. The reduced viability of the *recA* strain was taken into account as described in MATERIALS AND METHODS.

TABLE 7

Lysogen formation by mini-Mu phages injected by P22 virions

Recipient strain no.	Genotype of recipient	Transposition functions in recipient[a]	No. of Mu*d*F lysogens (Kn^r transductants)	Relative frequency of lysogens
TT10377	*rec*$^+$	Mu*A*	2400	1
TT8353	*rec*$^+$	Mu*A* and *B*	30	0.01

Recipient strains were grown overnight in complex medium containing ampicillin (50 μg/ml). A P22 transducing lysate grown on strain TT12116 was used to infect recipient cells at a multiplicity of 2. The experiment was performed at 37°.

[a] Plasmids expressing either Mu*A* or Mu*A* and *B* functions were obtained from P. VAN DE PUTTE.

there is an almost 20-fold reduction in the recovery of mini-Mu lysogens and only about a 3-fold reduction in recovery of Ap^R lysogens. Although both genomes are capable of killing and both possess normally regulated transposition function, only the mini-Mu*d* is strongly affected by the recipient *rec* mutation.

We will suggest later that the *rec* effects described above are due to killing of the host by chromosome breaks induced by replicative transposition events which occur when Mu fails to transpose conservatively. We suggest that the default replicative transposition breaks the target chromosome, leaving one copy of the Mu element at each end of the broken chromosome. The resulting breaks can be repaired by *rec*-mediated repair events. In *rec* mutant hosts this route of rejoining is impaired and many potential lysogens are lost.

A *rec*-independent route of chromosome rejoining is by a secondary replicative transposition of either copy of the Mu element into sequences near the opposite end of the broken chromosome; this would rejoin the broken ends and would cause a deletion adjacent to the site of the single remaining Mud element. We have looked for evidence of the predicted deletions.

Deletions are more frequently associated with insertions in Rec$^-$ recipients: It is well known that insertion of phage Mu is occasionally associated with formation of a deletion of target material adjacent to the insertion site (DANIELL, ROBERTS and ABELSON, 1972; HOWE and ZIPSER, 1974; CABEZON *et al.* 1978; HOWE and SCHUMM, 1981). These deletions are presumed to be due to secondary replicative transposition events occurring before the prophage is fully repressed. We have observed an increase in the frequency of adjacent deletions in the lysogens formed by Mu*d* elements in situations that are subject to the *rec*-effect described above. That is, the frequency of deletions adjacent to the integration site increases among the reduced number of lysogens recovered using *rec* recipients.

Increased deletion formation is demonstrated in an experiment in which phage P22 transduces both Mu*d*I(Ap) and Mu*d*J(Km) from a single donor strain into a recipient which cannot supply transposition functions. The donor strain carries Mu*d*I and Mu*d*J insertions close together within the histidine operon; the transposition-proficient Mu*d*I element is oriented with its Mu *A* and Mu *B* genes closest to the nearby

TABLE 8

Effect of a *recA* mutation on the relative frequency of lysogens of two transposition proficient Mu derivatives, mini-Mud (MudII1681) and Mu*p*API

Recipient strain no.	Recipient relevant genotype	No. of MudII1681 (Km^R) lysogens (adjusted)[a]	Relative frequency of mini-MudII lysogens	No. of Mu*p*Ap1 (Ap^R) lysogens (adjusted)[a]	Relative frequency of Mu*p*Ap1 lysogens
TT10502	*rec*$^+$	1859[b]	1	8497[b]	1
TT10503	*recA*	105	0.06	2942	0.35

Recipient strains were grown overnight in complex medium at 30°. The dilysogenic strain (TT17337) was induced and transductions were performed as described by BERMAN, ENQUIST and SILHAVY (1981). The multiplicity of infection was 10^{-3}. The single suspension of infected cells was plated onto nutrient broth medium and incubated under nonselective conditions for 20 hr. Lysogens of Mu*d*II1681 and Mup*Ap1* were detected by replica-printing the lawn of plated, infected cells sequentially onto medium containing either ampicillin or kanamycin.

[a] All of the Km^R MudII1681 lysogens formed by transposition since none of the Km^R transductants inherited the donor Ara^- phenotype; transductants arising by homologous recombination would be expected to show this donor phenotype.

[b] Numbers of transductants arising in the *recA* strain have been adjusted to account for the reduced viability of these strains. The relative viability of *recA* strains is 0.59 compared to that of an isogenic *recA*$^+$ strain; number of recombinants has been increased by dividing the number of counted transductants by 0.59.

Mu*d*J insertion. Transduced fragments that include Mu*d*J frequently include the Mu *A* and *B* genes of the Mu*d*I element and thus are provided with regulated transposition functions located *cis* to the Mu*d*J element. This donor strain has been described previously (HUGHES and ROTH 1988). In this experiment, transposition functions are supplied to the defective Mu*d*J element by normal, regulated Mu *A* and *B* genes of the Mu*d*I element, present on the same transduced fragment. To test Mu*d*I transposition, P22 transducing lysates were grown on strain TT7216, which has only the *his*::Mu*d*I insertion and does not carry a Mu*d*J element.

To detect deletion mutants among transposition transductants, selection was made for either Km^R (Mu*d*J) or Ap^R (Mu*d*I) transductants (lysogens) on MacConkey galactose plates. On these plates, lysogens that have acquired an insert (or a deletion) within the galactose operon form white colonies while transductants with an intact galactose operon form red colonies. By scoring the nutritional phenotypes of Gal^- transductants, one can identify deletion mutants that are also defective for either or both of the loci (*nadA* and *bio*) which flank the galactose operon. This is a minimal estimate of deletion formation since it only detects deletions long enough to damage at least one of these nearby loci.

The frequencies of deletions observed in this experiment are presented in Table 9. The first section presents the results when Mu*d*J is transduced by P22. In *rec*-deficient recipients, the total number of Km^R transductants is reduced (5-fold lower in *recA* or *recB* strains and 50-fold lower in *recA recB* strains; data not shown) and the frequency of deletions is increased. The majority of the deletions (6/7 tested) exhibit 100% linkage with the Mu*d*J; supporting their being formed by the integration event. The second section of Table 9 includes results seen when the larger Mu*d*I element is transduced by P22. As for Mu*d*J, *rec* recipients show a lower total number of Ap^R transductants (unpublished results; and HUGHES, OLIVERA and ROTH 1987) and the fraction of insertions having adjacent deletions is increased.

The third section of Table 9 presents the behavior of the same Mu*d*I element when introduced by a Mu virion. This was achieved by constructing a donor strain (TT8050) which carries both Mu*d*I and a Muc^{ts} prophage. At high temperature, a mixed lysate can be made that includes both Muc^{ts} genomes and Mu*d*I genomes, within Mu capsids. This lysate was used to transduce recipient strains that are sensitive to Mu infection by virtue of a *mus* mutation (FAELEN *et al.* 1981). Two points should be noted. First, the frequency of Ap^R transductants is affected very little by the presence of a *rec* mutation in the recipient (HUGHES, OLIVERA and ROTH 1987; data not shown). Second, the frequency of deletions is low and there is no evidence for an increase in deletion frequency in the *rec* recipient strains.

DISCUSSION

Under several conditions, host *rec* mutations cause a reduction in the ability of derivatives of phage Mu to form lysogens. We propose that the reduction in lysogen recovery is due to phage-induced killing which can be avoided in cells having normal recombination functions. More specifically we suggest that the phages showing a *rec* effect all share a defect in performing Mu's initial conservative transposition. Because of this defect, they transpose replicatively from the linear injected genome; this causes a chromosome break, which is a potentially lethal but repairable damage.

Normally a newly injected Mu genome is thought to transpose conservatively into the host chromosome.

TABLE 9

Frequency of extended deletions among Gal⁻ insertion mutants

Genotype of recipient	Mud*J* introduced by P22 particles[a]			Mud*I* introduced by P22 particles[a]			Mud*I* introduced by Mu particles[a,b]		
	Donor and recipient[c]	Frequency of Gal⁻ colonies[d]	Frequency of deletions among Gal⁻ colonies[e]	Donor and recipient[f]	Frequency of Gal⁻ colonies[d]	Frequency of deletions among Gal⁻ colonies[e]	Donor and recipient[f]	Frequency of Gal⁻ colonies[d]	Frequency of deletions among Gal⁻ colonies[e]
rec⁺	TT10288 and LT2	0.1% (35/35000)	8.6% (3/35)	TT7216 and LT2	0.037% (29/78378)	13.8% (4/29)	TT8050 and TT10502	0.12% (40/33330)	5% (2/40)
recA	TT10288 and TT14555	0.35% (29/8260)	27.6% (8/29)	TT7216 and TT14555	0.26% (28/10769)	60.7% (17/28)	TT8050 and TT10503	0.18% (37/20558)	0% (0/37)
recB	TT10288 and TT14556	0.37% (20/5333)	50% (10/20)	TT7216 and TT14556	0.5% (18/3600)	66.7% (12/18)	TT8050 and TT10504	0.28% (40/14284)	7.5% (3/40)
recA recB	TT10288 and TT14558	0.33% (15/4540)	60% (9/15)	TT7216 and TT14558	0.098% (16/16326)	75% (12/16)	TT8050 and TT10505	0.1% (40/39997)	0% (0/40)

[a] The multiplicity of infection was between 1 and 4.
[b] To permit infection by phage Mu, both donor and recipient strains carry a *mus* mutation.
[c] The experiment was performed at 37°.
[d] The numbers in parentheses indicate the number of Gal⁻ colonies among the total number of Mu*d* lysogens scored. The total scored does not reflect the frequency of Kn^R transductants. Since survival of transductants is extremely low in *recB* and *recA recB* strains many more infected cells were screened to accumulate sufficient transductants [see frequencies in Table 2 and HUGHES, OLIVERA and ROTH (1987)].
[e] The numbers in parentheses indicate the number of extended gal deletions among the total number of Gal⁻ colonies scored.
[f] The experiment was performed at 30° to prevent lytic growth of MudI.

This leaves a cell with an intact bacterial chromosome which can survive as a lysogen (if no subsequent transposition occurs) or can lyse following a series of secondary rounds of replicative Mu transposition. If the initial conservative transposition event were prevented, and a replicative transposition occurred from the injected linear molecule, a break would be caused in the host chromosome, leaving one copy of the Mu prophage at each broken end (see Figure 2). Unless repaired, such breaks would be lethal. Repair could occur by homologous recombination between the two Mu genomes to regenerate a complete circular host chromosome containing a single added Mu prophage. Such repair would be stimulated by the host *recA, B* and *C* functions. We suggest that under some of the conditions of Mu infection described here, the infecting Mu genome is *unable* to transpose conservatively. Replicative transposition, we suggest, then occurs, inducing a break in the host chromosome. Under these conditions, a *rec*-deficient host shows a decrease in the frequency of surviving lysogens.

In the absence of *rec* functions, the Mu-induced chromosome breaks described above can be rejoined if one of the Mu prophages at the chromosome break points performs a second replicative transposition to a target site near the opposite chromosome end. If strand exchange during transposition occurs in one of the two possible orientations and the target site is sufficiently close to the broken end, this transposition event would reform the circular host chromosome and leave a viable strain with a deletion of chromosomal material adjacent to the added Mu prophage at the site of Mu insertion (Figure 2). We propose that in a *rec* mutant host a larger fraction of recovered lysogens would be formed by this secondary transposition event. The predicted increase in the frequency of deletion mutants was found among the lysogens recovered in *rec*-deficient hosts.

Reduced lysogen recovery in hosts with *rec* mutations (due, we argue, to the failure of conservative transposition) was shown by Mu derivatives under several circumstances (Figure 3). Sensitivity to a host *rec* defect was seen for all tested Mu derivatives when their genome was injected by a virion of phage P22; this included both transposition-proficient Mu derivatives and defective elements whose transposition function was provided by a plasmid carrying the Mu *A* gene. Mini-Mu phages showed a *rec* effect following injection by either Mu or P22 virions. Lysogeny by Mu*d*I elements as well as the plaque-forming Mu derivative, Mu*p*ApI (and presumably wild-type phage Mu), is unaffected by host *rec* mutations when these elements are injected by a Mu virion.

The fact that genomes delivered by a P22 virion are subject to *rec* effects suggests that a function (presumably a protein), normally injected by the Mu virion, cannot be provided by the P22 virion and thus cannot prevent the *rec* sensitivity. Since mini-Mu*d* phages show *rec* sensitivity regardless of the injecting virion, we suggest that they may lack or improperly position some part of the Mu genome required for injection or action of this protein. It is interesting to note that in experiments with mixed infection of Mu*p*ApI and Mini-Mu*d* (Tables 5 and 8), these two

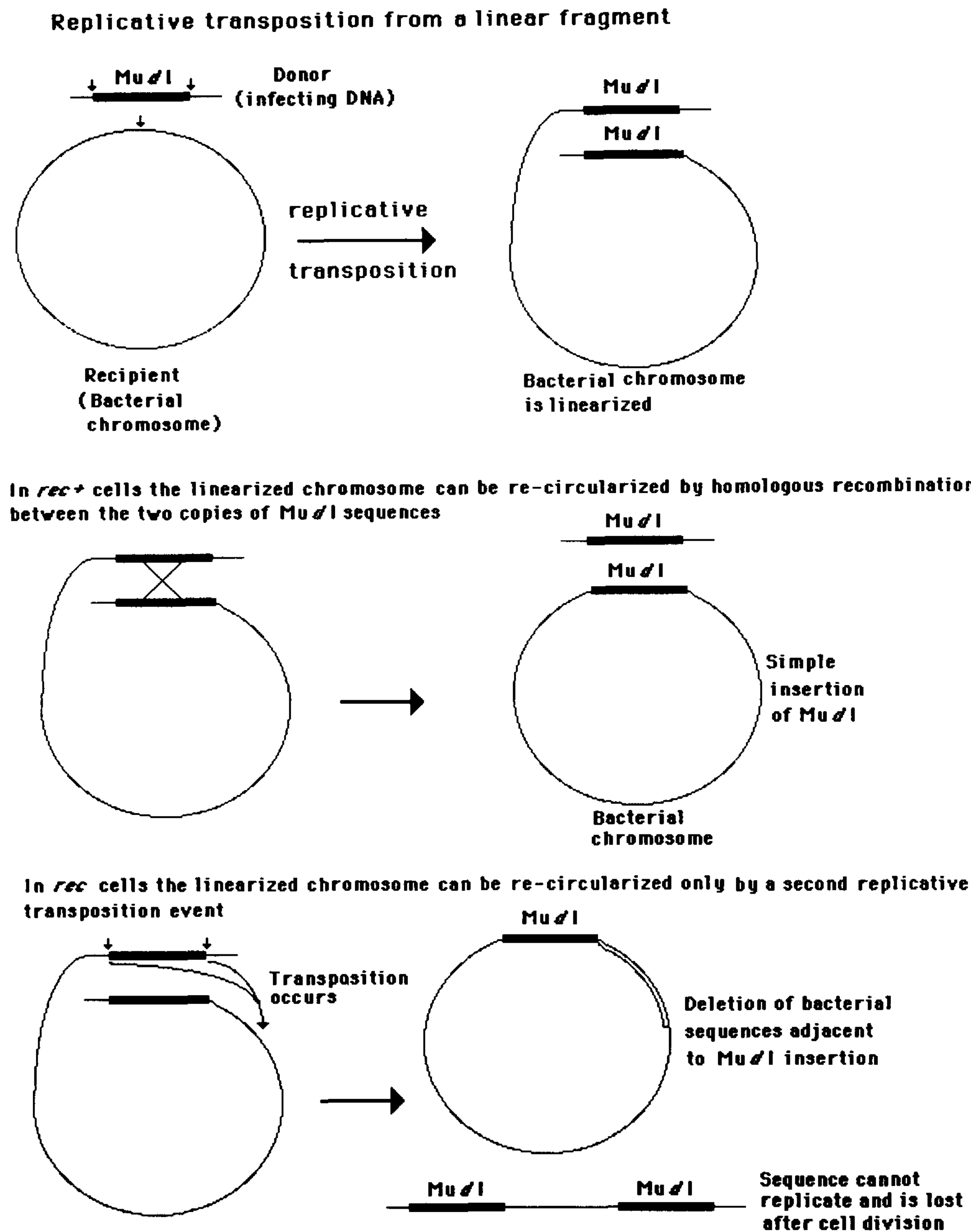

FIGURE 2.—Consequences of replicative transposition of a P22 packaged Mu*d*I element onto the bacterial chromosome. (1) The bacterial chromosome is broken during replicative transposition of Mu*d*I from a P22 transduced fragment. (2) In *rec*+ cells the broken chromosome can be re-circularized by homologous recombination between the Mu*d*I elements. (3) In *rec* cells the broken chromosome can be re-circularized by a secondary transposition event involving one of the Mu*d* elements. The genetic material between the Mu*d* insertion and the target site is deleted. The small arrows represent sites at which Mu transposase introduces either single strand nicks or double strand breaks in DNA.

genomes are replicated and packaged into Mu virions by the same donor cells. The progeny phages inject into the same host cell and (at high helper multiplicity) transposition functions are provided in *trans* to the defective Mu derivative (Table 5). Nevertheless, the frequency of mini-Mu*d* lysogens is reduced in *rec*-deficient hosts. This suggests that a plaque-forming Mu*p*ApI genome cannot provide, *in either the donor or recipient cell,* the function needed to protect mini-Mu*d* phages from Rec effects. This leads us to suggest that mini-Mu*d* phages lack genomic features required either for injection or action of the protein that promotes conservative transposition. We predict that normally the protein is tightly associated with the injected genome and cannot act in *trans* to aid other genomes in the new host.

The function hypothesized above shares many features with a known Mu protein that has been previously studied in considerable detail. The Mu *N* gene encodes a Mu virion protein that is injected into the recipient cell along with the Mu DNA (GLOOR and CHACONAS 1986). The Mu *N* gene is one of the late genes of Mu and was originally identified as being essential for the synthesis of Mu tails (GRUNDY and HOWE 1985). Besides its role in tail formation, the Mu *N* gene product can protect transfecting Mu DNA from degradation by exonuclease V (CHASE and BENZINGER 1982; and HARSHEY and BUKHARI 1983). Consistent with the ability to recognize a specific site in DNA is the fact that the amino acid sequence of the *N* protein includes a helix turn helix motif common

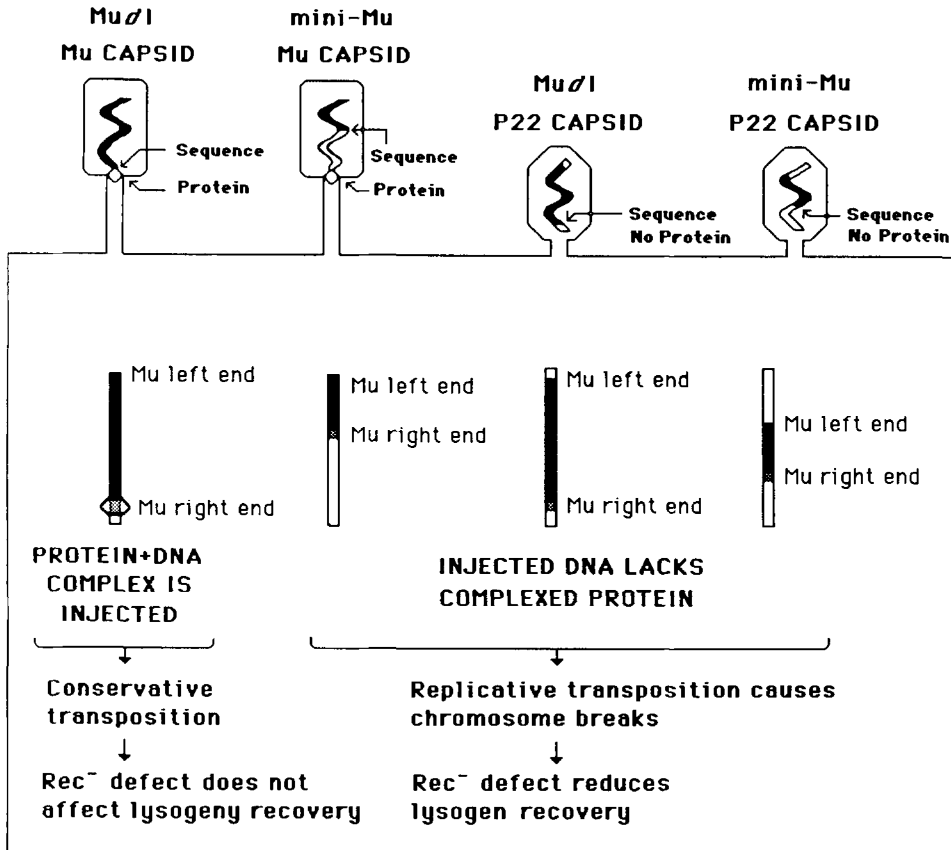

FIGURE 3.—Model to explain the effect of the packaging virion on the recovery of Mu*d* phage lysogens. In the figure solid black genome segments indicate Mu sequences; adjacent while sequences are chromosomal sequences. The small shaded sequence is a hypothesized binding site for the injected protein. It is proposed that a Mu-encoded tail protein normally associates with a specific sequence at the right end of the Mu genome during phage assembly and is injected in association with that sequence. Once injected, the protein contributes to conservative transposition of the Mu or Mu*d* genome. This protein can be injected by Mu virions which carry a Mu or a full sized Mu*d* genome; in mini-Mu phages (due to headful packaging) the binding site is located within the capsid and cannot associate with the tail protein. An alternative model (not depicted) is that the critical sequence is missing from the mini-Mu phages tested. In either model, P22 virions do not inject the needed protein. Without this injected protein, the initial transposition event is replicative and causes a break in the recipient chromosome.

to many regulatory proteins (GLOOR and CHACONAS, 1988). Once bound to DNA, *N* protein cannot be transferred to another DNA molecule in vitro (G. GLOOR and G. CHACONAS, personal communication). In recipient cells, the Mu *N* gene product circularizes the infecting DNA by binding at or close to the ends of the DNA in a non-covalent fashion (HARSHEY and BUKHARI 1983; PUSPURS, TRUN and REEVE 1983; and GLOOR and CHACONAS 1986). Most importantly, it has been suggested that this protein plays a role in conservative transposition (GLOOR and CHACONAS 1986). These properties of the Mu *N* gene product suggest it is the function that facilitates recovery of Mu*d* lysogens in Rec⁻ strains.

If the Mu *N* protein explains the results presented here, our results, although indirect, may shed light on several aspects of the function and mode of injection of the Mu *N* protein. If the defect in conservative transposition of mini-Mu genomes (packaged in a normal Mu virion) is due to a lack of *N* protein, these phages must be defective in either injection or response to the Mu *N* function. This defect could reflect sequences missing from the mini-Mu*d* genome or, more likely, sequences that are improperly positioned in the mini-Mu virion. (Figure 3 describes the latter possibility.) The right end of a normal Mu genome is known to be associated with the Mu tail (INMAN, SCHNOS and HOWE, 1976; BREEPOEL *et al.* 1976). Because of Mu's headful packaging mechanism which starts at the left end of the genome, a mini-Mu with a small genome would have its right end located within the capsid unassociated with tail proteins. If the *N* protein must associate with the right end of Mu in the course of phage assembly, the location of the right end within the capsid of mini-Mu*d* phages would prevent *N* protein association and result in a failure of *N* protein to be injected into the new host.

The observations reported here point out a potential danger and a useful property of mini-Mu*d* phages when used in genetic analysis. These phages have an increased probability of generating deletions at the site of insertion, particularly when insertions are isolated in *rec*-deficient hosts (see Table 9). This can cause a problem when fusion insertions are formed to study gene regulation. The phenotype by which an insertion mutant is isolated could reflect material removed by an adjacent deletion and the gene or operon fusion providing *lac* expression may involve an unrelated gene at the end of the deletion. The propensity of mini-Mu phages to make deletions in *rec*-deficient hosts may be a valuable means of generating deletions since the frequency of deletions among surviving lysogens approaches 100%; these deletions are particularly valuable since they are associated with the select-

able drug resistance determinant of the mini-Mu*d* phage and thus can be introduced selectively into new genetic backgrounds.

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