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Stimulation of Deletions in the *Escherichia coli* Chromosome by Partially Induced Muc*ts*62 Prophages

M. FAELEN* AND A. TOUSSAINT

Laboratoire de Génétique, Département de Biologie Moléculaire, Université Libre de Bruxelles, B-1640 Rhode St. Genèse, Belgium

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Deletion of bacterial DNA fragments is stimulated in induced Muc*ts*62 lysogens. The host genes located proximally to the prophage are more frequently lost than those which are unlinked to the Mu genome. Genes located on either side of a Mu genome are deleted in the same manner. Like the other Mu-induced rearrangements, this process is *recA* independent and requires the participation of Mu DNA, as indicated by the fact that a phage genome always replaces the deleted genes. Data are presented which strongly suggest that both ends of the Mu genome are involved in deletion formation.

The temperate phage Mu-1 is very prone to integration within other DNAs. During its lytic cycle, it integrates at random in the chromosome, the plasmids, or other prophages of its host, and even Mu DNA extracted from viral particles consists of a phage genome integrated into a small piece of host DNA (for review, see 4).

Integration of Mu requires one site on its own genome and the product of the phage gene *A*. The target site used for integrative recombination seems to result from some kind of as yet unidentified interaction(s) between the ends of the Mu genome.

When infecting a sensitive host, or after induction, Mu can also stimulate rearrangements in the chromosome of its host: it causes fusions with other circular DNAs where the newly integrated DNA is surrounded by two Mu genomes in the same orientation (8, 9, 31, 33); translocations, where translocated DNA fragments are sandwiched between two Mu prophage similarly oriented (10, 11); and inversions, where the inverted sequences are surrounded by two Mu's in opposite orientation (Faelen et al., submitted for publication). The presence of Mu DNA at the site of the rearrangement shows that Mu DNA directly participates in these illegitimate recombination events, which are all Mu gene *A* dependent and *recA* independent (7, 31).

In this paper we describe the properties of Mu-induced deletions. It is already known that infecting Mu can stimulate the formation of deletions in the host chromosome and that a Mu prophage is inserted at the site of the deletion (6; M. Howe and D. Zipser, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V208, p. 235). We studied the properties of deletions generated by an induced Mu prophage and tried to determine which sites on Mu DNA are necessary to enable Mu to mediate the formation of deletions.

MATERIALS AND METHODS

Bacteriophages. The following phages were used: Muc$^+$ (29); Muc*ts*62 (14); Muc*ts*62*X*: Muc*ts*62*B*::IS*1* (3, 5); MPh1: Muc*ts*62 with P1CM host range (Toussaint et al., submitted for publication); ϕ80c$^+$ (24); ϕ80hycI (28); ϕ80*vir* (Maurer-Richelle); λc^+ (19); λcI (16); λcI857 *recA* (23); $\lambda i21c^+$ *att*ϕ80 (this laboratory); derivatives of phage λi434 carrying the amber mutations *Psus*3, *Qsus*203, *Rsus*216, *Fsus*204, *Ksus*24, and *Jsus*6 (30); λc^+702, c^+ derivative of a λcI857 carrying the *c* end of a Muc*ts*62 genome (Howe); λc^+443, c^+ derivative of a λc*I*857 carrying the *S* end of a Muc*ts*62 genome (Howe); $\lambda i21$cI::Mu40, $\lambda i21$ derivative carrying a Mu40 prophage in its cI gene; f2 (22); Mu40, mini-Mu derived from a Muc*ts*62A::Tn*9* prophage, which is deleted from genes *A* to *S* inclusive (Faelen et al., submitted for publication).

Bacteria. Bacterial strains used are listed in Table 1.

Media. L broth (20) was used for the cell cultures. Bacteria and Mu and lambdoid phages were titrated on L agar. Bacterial dilutions were made into 10^{-2} M $MgSO_4$; phages were diluted in SM buffer (34). Factors required by the cells to grow were determined on suitably supplemented minimal medium 132 (13). Soft agar was T broth (16) with 0.7% agar. Gal^r Chl^r deletions were selected on McConkey agar containing 1% galactose and 0.2% $KClO_3$.

Selection of *tonB* mutants. Cultures of the bacteria to be tested were grown overnight in L broth at 37°C without shaking. One loopful of each culture was diluted in 5 ml of L broth and grown up to saturation at 37°C. Viable bacteria were titrated on L agar at 30°C; *tonB* clones were isolated by adsorbing 0.2 ml of

TABLE 1. *Bacterial strains used*

Strain	Genetic markers	Origin, derivation, or reference
RH3701	F⁻ Δ(*lac-pro*) *thi rpsL recA* (φ80*c*⁺)	CSH54 (25)
RH3702	Same as RH3701 except *trp*::(−Mu*cts*62)	RH3701
RH3703	Same as RH3701 except *trp*::(+Mu*cts*62)	RH3701
RH3704	Same as RH3702 except (Muc⁺)	RH3702
RH3705	Same as RH3703 except (Muc⁺)	RH3703
RH3706	F⁻ Δ(*lac-pro*) *galE thi sup*⁺ *recA*	CSH41 (25)
RH3707	RH3706 (λ*c*⁺)	RH3706
RH3708	RH3706 (λ::(Mu*cts*62)*c*⁺)	RH3706
RH3709	RH3707/F*lacI*::(Mu*cts*62) *proA,B*[a]	RH3707 × Bu8220 (Bukhari)
RH3710	RH3707/F*lacZ*::(Mu*cts*62*X*5001) *proA,B*[a]	RH3707 × Bu8564 (Bukhari)
RH3711	RH3706 (λ*c*⁺702)/(F*lacZ*::(Mu*cts*62*X*5001) *proA,B*[a]	RH3706
RH3712	RH3706 (λ*c*⁺443)/F*lacZ*::(Mu*cts*62*X*5001) *proA,B*[a]	RH3706
RH3713	RH3706 (λ*i*21c*I*::Mu40) (λ*i*21*c*⁺ φ80*att*)/ (F*lacZ*::(Mu*cts*62*X*5001) *proA,B*[a]	RH3706
594	F⁻ *lac gal-1 gal-2 rpsL sup*⁺	30
594 (Muc⁺)	Same as 594 except (Muc⁺)	
C600	F⁻ *thr leu thi lacY tonB supE*	1
C600 (Muc⁺)	Same as C600 except (Muc⁺)	
CSH33	*thi*/FColV,B *trp cys*	25
M5020G	F⁻ Δ(*lac-pro*) *rpsL*	

[a] These episomes derive from F128 (see reference 25).

a φ80*vir* lysate (10^{11} phages per ml) and 0.2 ml of a colicin (Col) V,B suspension (26) to 0.1 ml of a 10^{-1} dilution of the bacteria. After incubation for 20 min at 30°C, surviving cells were assayed at 30°C on L agar plates seeded with 0.1 ml of both the φ80*vir* and the ColV,B lysates.

Selection of Gal^r Chl^r mutants. Cultures of cells with a *galE* mutation were centrifuged and resuspended in 0.2 ml of 10^{-2} M $MgSO_4$; 0.1 ml of these suspensions, or of diluted bacteria, was plated on selective medium. The plates were placed in a GasPak anaerobic jar with three hydrogen-plus-CO_2 generator envelopes, and, after addition of 10 ml of water to each envelope, the jar was hermetically sealed and incubated at 35°C for 24 h. The plates were then incubated aerobically for a further 24 h at 35°C.

Analysis of *tonB* mutants. *tonB* clones were purified at 30°C by streaking onto L agar plates seeded with 0.2 ml of the ColV,B lysate and further streaking onto L agar. Isolated clones were resuspended in drops of 10^{-2} M $MgSO_4$. Production of φ80*c*⁺ and Mu*cts*62 phages was assayed at 37°C on lawns of either strain 594 (Muc⁺) or strain C600. φ80 immunity was tested by cross-streaking the mutant with phage φ80hy1c*I* at 30°C. The presence of the *cysB* gene was tested by spotting the cells on minimal medium supplemented with glucose (1%), sodium citrate (0.05 M), and the factors required for growth of the *tonB*⁺ parental strain. The *rec*⁺ allele was introduced by mating the cells with Hfr KL16 and selecting for UV^r Sm^r recombinants on L agar plates containing streptomycin (100 μg/ml). These *rec*⁺ derivatives were transduced to *trp*⁺ with phage P1 according to the procedure of Lennox (20), and the *trp*⁺ clones were tested for the *tonB* marker and the Mu*cts*62 prophage.

Analysis of Gal^r Chl^r mutants. Isolated Gal^r Chl^r clones grown at 30°C were resuspended in drops of 10^{-2} M $MgSO_4$. Production of Mu*cts*62 particles was assayed by spotting the cells on a lawn of C600 (λ*c*⁺) at 37°C. Lambda immunity was tested by cross-streaking the cells with phage λ*c*I at 30°C. Mu immunity was assayed by spotting dilutions of MPh1 onto lawns of the bacteria to be tested and incubating the plates at 30°C. The presence of the *bio, nad,* and *suc* genes was examined by spotting the mutants onto minimal medium supplemented with glucose (1%), proline (80 μg/ml), and biotin (1 μg/ml), nicotinic acid (1 μg/ml), or succinate (80 μg/ml), or different combinations of these three substances. Deletion of *aroG* was investigated by testing the cells on minimal medium supplemented with tryptophan (80 μg/ml), tyrosine (80 μg/ml), and the various factors required for growth of the mutated bacteria. The test for the *pgl* gene was made according to Kupor and Fraenkel (18). Loss of λ prophage genes was investigated by the marker rescue test (12): dilutions of amber (Am) mutants of the λ*i*434 were spotted onto lawns of either the *gal chl sup*⁺ bacteria or strain 594 (a *sup*⁺ λ⁻ control) at 30°C. Lysis in the spot on a *gal chl sup*⁺ mutant but not on strain 594 indicated that the Am⁺ allele of the λ*i*434 phage mutant was still present in the strain. Rec⁺ derivatives of the mutants were isolated by lysogenizing the cells with λc*I*857*recA*⁺ at 30°C and selecting for UV^r clones at 37°C. The Rec⁺ cells were transduced to Gal⁺ with phage P1 and tested for the inheritance of the markers linked to Gal and the loss of their Mu prophage.

When derived from strains with a Mu genome in the F *lac pro* episome, the Gal^r, Chl^r mutants were first tested for the presence of a Mu genome in the episome by looking for the cotransfer of the Mu prophage with the *pro* genes to a Δ (*lac-pro*) Sm^r recipient at 30°C. F⁻ Pro⁻ derivatives of the mutants were isolated among the survivors of an infection with the male-specific phage f2 and analyzed as described above.

RESULTS

***tonB* deletions induced by a Mu*cts*62 prophage inserted in the *trp* operon.** Deletions

in the *tonB-trp* region were selected in a Mu-sensitive strain and in four different Mu lysogens. All were *recA* and φ80 lysogens. Two of the Mu lysogens had a unique Muc*ts*62 prophage inserted in the *trp* operon in either the negative (RH3702) or the positive (RH3703) orientation. Strains RH3704 and RH3705 also contained a Muc$^+$ prophage, thereby preventing the thermal induction of Muc*ts*62. Bacteria resistant to φ80*vir* and ColV,B were selected after growing the cells at 37°C. Under these conditions the prophage is partially induced, and about 50% of the induced bacteria survive the thermal induction (10). Table 2 shows that the Mu single lysogens gave roughly 100 times more resistant clones than either the Mu-sensitive control or the derivatives with a Muc$^+$ prophage. Some resistant colonies generated by RH3702 and RH3703 were purified for analysis. None of them produced φ80 particles (see Table 3), although some of these clones retained the φ80 immunity. Since the φ80 genome was inserted close to the left side of the *tonB* gene in the parental strain, we concluded that the mutants were indeed deletions of the *tonB* region extending at least into the φ80 prophage, some of them having the φ80 immunity region intact and others not. These deletions did not extend into the *cysB* gene, which is located on the opposite side of the *trp* operon (2). All of these mutants still produced Muc*ts*62 particles upon induction. Location of the prophage in the *tonB-trp* area was determined as described in Materials and Methods; when transduced to *trp*$^+$, the deleted clones regained sensitivity to φ80*vir* and lost their Muc*ts*62. Therefore, the Muc*ts*62 genome must be closely linked to both the *trp* operon (as is the Muc*ts*62 prophage in the parental strain) and the deletion. Thus, *tonB* deletions isolated from strains having a Mu in the negative or positive orientation in *trp* are of the same type and seem to always have one end located between the *tonB* gene and the genome of the Muc*ts*62 prophage. We previously described the stimulation of *tonB* deletions by a Muc*ts*62*X* prophage inserted in the *trp* operon (7). Analysis of 10 different Mu-induced *tonB* mutants derived from such a lysogen shows that all retain the bacterial genes to the right of the *tonB-trp* area and a complete Mu genome, since a few Mu*X*$^+$ revertants could be recovered after induction of these cells. Whereas the parental Muc*ts*62*X* prophage could be excised precisely to give *trp*$^+$ revertants, none of the deleted clones reverted to *trp*$^+$, probably reflecting the loss of that part of the *trp* operon located between *tonB* and the Mu genome. This would locate one extremity of the deletions within the *trp* operon, probably at one extremity of the Mu prophage.

Mu-induced deletions of the *gal-chlD* region. The *gal* operon and the *chlD* genes of *Escherichia coli* K-12 are near the λ attachment site (attλ) on the bacterial chromosome (2). Simultaneous loss of the *gal* and *chlD* functions was stimulated by growing at 37°C *recA* lysogens carrying a Muc*ts*62 prophage inserted between genes *F* and *K* of a λ prophage (RH3708, Table 4). Some of these Gal^r Chl^r clones were characterized and were shown to have lost the bacterial *pgl* gene and the λ genes *cI*, *P*, *R*, and *F* (see Fig. 1). Some of them retained the *aroG* and *nadA* genes, whereas others showed an Aro$^-$ or Aro$^-$ Nad$^-$ phenotype. All clones still contained the λ genes *K* to *J* and a complete

TABLE 2. *Stimulation of tonB mutant formation in Muc*ts*62 lysogens*[a]

Strain	Mu prophage(s) present in strain	Frequency of *tonB* clones
RH3701		1.7×10^{-6}
RH3702	*trp*::(−Muc*ts*62)	3.7×10^{-4}
RH3703	*trp*::(+Muc*ts*62)	2×10^{-4}
RH3704	*trp*::(−Muc*ts*62) (Muc$^+$)	1.8×10^{-6}
RH3705	*trp*::(+Muc*ts*62) (Muc$^+$)	1.4×10^{-6}

[a] *tonB* clones were selected as described in the text. The frequency of *tonB* clones is given as the ratio, number of clones resistant to φ80*vir* and ColV,B per milliliter/number of bacteria per milliliter.

TABLE 3. *Analysis of tonB clones derived from strains RH3702 and RH3703*[a]

Origin of the *tonB* clones tested	No. of clones/total:			No. of cys$^+$ clones/total
	No. Producing φ80	No. Showing φ80 immunity	No. Producing Mu	
RH3702	0/60	21/60	60/60	60/60
RH3703	0/60	16/60	60/60	60/60

[a] Purified Mu-induced *ton*B clones were tested as described in the text.

TABLE 4. *Stimulation of Gal^r Chl^r mutant formation in Muc*ts*62 lysogens*[a]

Strain	Prophage(s) present in strain	Frequency of Gal^r Chl^r clones
RH3707	λ*c*$^+$	4.5×10^{-9}
RH3708	λ::(Muc*ts*62)	5×10^{-4}
RH3709	λ*c*$^+$ *lacI*::(Muc*ts*62)	2×10^{-5}

[a] Gal^r Chl^r clones were selected as described in the text. RH3708 contains a Muc*ts*62 prophage inserted between the *F* and *K* genes of a λ*c*$^+$ prophage (see Fig. 1). RH3709 carries an F*lac pro* episome with a Muc*ts*62 prophage inserted in the *lacI* gene. The frequency of Gal^r Chl^r clones is given by the ratio, number of clones resistant to *gal* and $KClO_3$ per milliliter/number of bacteria per milliliter.

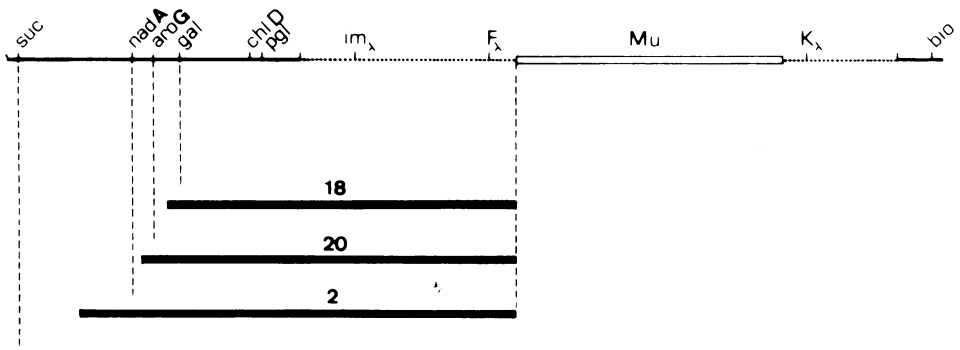

FIG. 1. *End points of Gal^r Chl^r deletions induced by a Mu inserted in a λ prophage. Gal^r Chl^r clones were tested as described in the text. Numbers over the heavy lines represent the number of clones of each type. The dashed line represents λ DNA; open line, Mu DNA; plain line, host DNA. The heavy lines represent the DNA segment missing in the Gal^r Chl^r clones. This drawing is approximately to scale.*

Mu*cts*62 prophage. When *rec*$^+$ derivatives of these Gal^r Chl^r were transduced to Gal$^+$, they became Chl$^+$ Pgl$^+$ (Aro$^+$ Nad$^+$) and lost their Mu*cts*62 prophage and the λ genes *K* to *J*, showing that they contained a single Mu*cts*62 genome tightly linked to the deleted region. The deletions never extended either into the Mu genome or into the λ genes located to its right. Furthermore, these Mu-induced rearrangements did not always remove the same bacterial genes on the left side of the *gal-chlD* interval.

Incidence of the prophage location on the frequency of deletion formation. In the two experiments described above, we looked for deletions of genes closely linked to the Mu prophage. We also tested the ability of a Mu prophage to mediate deletions in *trans*, by measuring the frequency of Gal^r Chl^r deletions in a strain lysogenic for λ*c*$^+$ carrying an F*lacI*::(Mu*cts*62) *pro* episome (RH3709, see Table 4). In these experiments, after growth at 37°C, Gal^r Chl^r clones were recovered at a frequency of 2×10^{-5}, i.e., about 10^3 times higher than in the Mu-sensitive strain but about 25 times lower than in the strain which carries the Mu*cts*62 in λ*c*$^+$. Forty Gal$^-$ Chl^r clones derived from RH3709 were purified and tested. They can be assigned to nine different classes according to their respective end points (Fig. 2). The deletions cover genes lying on either one or both sides of the *gal-chl* region. Comparison of the number of clones belonging to each class reveals that the highest proportion of deletions (50%) retained the genes located on the left side of the *gal* operon. This might reflect the presence of a hot spot for Mu-mediated deletion formation in that region. However, since our analysis did not determine whether these deletions had the same end point in the *gal-aroG* interval, this point would require further study to be confirmed. Some of the Gal^r Chl^r clones were cured of their F′ episome, and all were found to still produce Mu*cts*62 phages, showing that they contain a Mu prophage outside the episome. Location of that new prophage next to the deleted region was demonstrated by P1 transduction of the Gal$^+$ Chl$^+$ region in *rec*$^+$ derivatives of those clones; transduction of the *gal*$^+$ allele invariably resulted in the rescue of the deleted markers and loss of the Mu prophage. To test whether insertion of the Mu genome next to the deleted region was accompanied by the excision of the Mu*cts*62 prophage from the F′ episome, the episome of some of the deleted derivatives was mated at 30°C into a Δ(*lac-pro*) Sm^r recipient. Pro$^+$ Sm^r sexductants were tested for inheritance of the Mu*cts*62 genome. All episomes tested still carried an intact Mu prophage, showing that the deleted clones had conserved an intact Mu genome at the original location, besides the transposition of a prophage near the deleted region. In previous experiments we found that a Mu*cts*62*X* prophage generates deletions in *cis* at the same efficiency as a Mu*cts*62 (7), showing that Mu*X* expresses all the functions required for the induction of deletions. The ability of Mu*cts*62*X* to mediate deletion formation in *trans* was measured by looking for Gal^r Chl^r deletions in a strain lysogenic for λ*c*$^+$ carrying an F*lacI*::Mu*cts*62*X pro* episome. Results in Table 5 show that Mu*X* provokes deletions at 1/500 the frequency of a Mu*cts*62 located at the same place. However, bacteria deleted by the Mu*X* prophage presented properties similar to those isolated from the Mu*X*$^+$ lysogen, their deletions being of variable size, whereas the bacteria maintained the prophage in their episome and contained a second Mu genome next to the deletion. Since a Mu prophage is found at the site of the deletion, the deletion process must here also involve

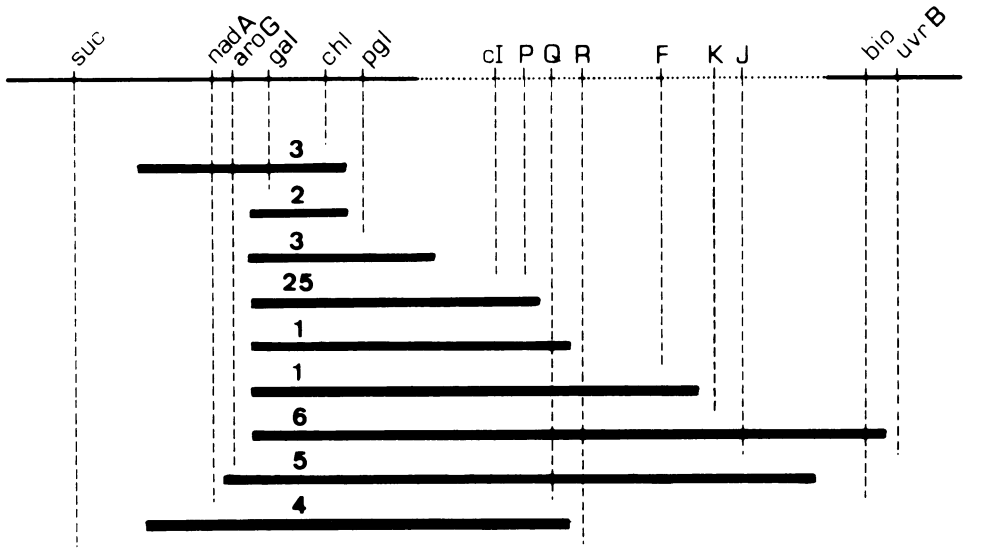

FIG. 2. *End points of Gal^r Chl^r deletions induced by Mu inserted in an F'lac pro episome. Symbols are the same as described in the legend to Fig. 1.*

TABLE 5. *Stimulation of Gal^r Chl^r deletions in strains with a Mucts62X prophage inserted in the lac operon of an Flac pro episome*[a]

Strain	Prophage(s) present in strain	Frequency of Gal^r Chl^r deletions
RH3707	λ*c*$^+$	5×10^{-9}
RH3710	λ*c*$^+$ *lacZ*::(Mu*cts*62*X*5001)	4.5×10^{-8}
RH3711	λ*c*$^+$702 *lacZ*::(Mu*cts*62*X*5001)	4×10^{-8}
RH3712	λ*c*$^+$443 *lacZ*::(Mu*cts*62*X*5001)	3×10^{-8}
RH3713	λ*im*21*c*I::Mu40 λ*im*21*c*$^+$ *att*φ80 *lacZ*::(Mu*cts*62*X*5001)	2.2×10^{-4}

[a] Gal^r Chl^r deletions were selected as described in the text. λ*c*$^+$702 contains the *c* extremity of a Mu*cts*62 genome; λ*c*$^+$443 contains the *S* extremity of a Mu*cts*62 genome; and λ*i*21*c*I::Mu40 contains the mini-Mu, Mu40, which is deleted from genes *A* to *S* but has intact *c* and *S* ends.

transposition of Mu DNA, which itself must require Mu replication. The fact that Mu*cts*62*X* mutants do not replicate (35) might therefore be an explanation for their decreased capacity to generate formation of deletions in *trans*.

Influence of a single Mu extremity on deletion formation. The transposition of Mu DNA involved in deletion formation most probably reflects the need for a site(s) of Mu DNA in the generation of deletions, rather than a need for Mu enzymes. Indeed, no *cis*-acting protein is known in Mu, and therefore any required enzymes could be provided in *trans*. The sites most likely to be involved in deletion formation are the extremities of the Mu genome. We therefore tested the influence of the presence of either one or the opposite extremity, or of both extremities, of Mu DNA, next to the region to be deleted, in a strain carrying a Mu*cts*62*X* prophage on an F' episome. The Mu*X* should in theory be able to provide for all necessary enzymes, since it mediates deletion formation in its vicinity with the same efficiency as a Mu*cts*62. Since none of these lysogens die at high temperature, in this case we looked for deletion formation in cultures grown at 42°C, where the two Mu prophages are fully induced. The frequency of Gal^r Chl^r deletions was measured in strains RH3711, RH3712, and RH3713, which, respectively, carry the *c* end, the *S* end, or both ends of Mu in a λ prophage. No increase in the deletion frequency was observed in either of those two strains carrying only one Mu end as compared with the control strain RH3710, which only has the Mu*cts*62*X* prophage on its episome (Table 5). However, the deleted prophage Mu40, which carries intact Mu ends, induces deletion formation about 5×10^3 times more frequently than the other deleted Mu tested. This strongly suggests that both extremities of the Mu genome are necessary to generate a deletion.

DISCUSSION

Genetic determinants in the vicinity of a gene sometimes affect the frequency at which that gene is deleted; IS elements (27) and transposons

(17) induce deletions of proximally located genes. Analysis of mutations induced by an infecting Mu indicates that integration of the phage genome can sometimes result in the loss of bacterial DNA in the vicinity of the Mu insertion site (Howe and Zipser, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V208, p. 235). The experiments we report here establish that partially induced Mu prophages increase the frequency of deletion of neighboring host genes; *tonB* deletions were stimulated at 37°C in lysogens with a Mu*cts*62 integrated in the *trp* operon. The orientation of the prophage inserted in *trp* did not affect the frequency at which the *tonB* gene was lost. Mu-induced deletions were not confined to the *tonB* area; stimulation of Gal^r Chl^r deletions occurred at 37°C in lysogens with a Mu*cts*62 integrated near *gal*.

When selection was made for the loss of bacterial genes located on one side of the Mu genome, the host gene located on the opposite side of the prophage always remained intact. In each case, the deleted clones retained a complete Mu genome which was tightly linked to the deletion. One end point of the deletions probably corresponds to the distal extremity of the prophage, whereas the opposite end point seems to be randomly distributed. A partially induced Mu*cts*62 prophage located on an episome can also generate deletions on the chromosome, although the frequency was about 10 times lower than in the case where the prophage was located near the genes to be deleted. In this case a Mu genome, which replaces the deleted region, is also found. The end points of the deletions seem to be randomly distributed in the region studied. One end probably corresponds to one extremity of the Mu prophage since the deletion never enters the Mu genome.

Since most of the strains used in this work were *recA*, it is clear that formation of a Mu-mediated deletion is a *recA*-independent process which has been shown to require the Mu *A* gene product (7). When the Mu was originally located on an episome in the parental strain, a Mu prophage was always found adjacent to the deletions, showing that transposition of Mu is involved in the induction of these deletions. This most probably reflects the need for sites on Mu DNA at the place where the deletion is formed. When transposition occurs, one copy of the Mu prophage is still present at the original location, as expected since no excision of the Mu genome can be detected upon induction of Mu lysogen (21). To define which sites on Mu DNA are essential for deletion formation, we tested whether the presence of a single Mu extremity, either the *c* end or the *S* end, was sufficient to stimulate deletion of *gal chl* genes located proximally. These defective Mu cannot synthesize the necessary enzymes. Therefore, the strains used also carried an F′ episome with an inserted Mu*cts*62*X* genome. In theory the Mu*cts*62*X* prophage could provide in *trans* all the necessary enzymes, since it can mediate deletion formation in its vicinity at a normal frequency. However, neither the *c* nor the *S* ends of Mu stimulate deletion of host genes to which they are linked. On the contrary, another deleted Mu prophage, Mu40, which carries intact ends but lacks all Mu functions essential for integration, replication, and the other steps of the lytic cycle, can generate deletions normally in the presence of a Mu*cts*62*X* helper. This strongly suggests, as we postulated previously (32), that both ends of Mu DNA are involved in the process of Mu-induced deletion formation.

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