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Model for the Enhancement of λ-*gal* Integration into Partially Induced Mu-1 Lysogens

MICHEL FAELEN,[1] ARIANE TOUSSAINT,* AND JEAN DE LAFONTEYNE[2]
Laboratoire de Génétique, Université libre de Bruxelles, B 1640 Rhode St. Genèse, Belgium

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Temperate phage Mu-1, which is able to integrate at random in its host chromosome, is also able to mediate integration of other circular deoxyribonucleic acid, as a λ-*gal* mutant unable to integrate by itself. After mixed infection with λ-*gal* and Muc$^+$, *gal*$^+$ transductants are recovered that have the λ-*gal* integrated in any circular permutation, sandwiched between two complete Mu genomes in the same orientation, the whole Mu-λ-*gal*-Mu structure being found at any location in the bacterial chromosome. Here we show that such a λ-*gal* can integrate in an induced Mu lysogen. In this case the λ-*gal* is again in any circular permutation, between two Mu in the same orientation, but it is always located at the site of the original Mu prophage, and the two surrounding Mu have always the same genotype as the original Mu prophage. Active Mu replication functions are not essential for that process to occur. This suggests that bacterial replication may generate two Mu copies that in some way can regenerate a Mu attachment site that recombines with the λ-*gal*. A model is presented that accounts for these observations, may be helpful for understanding some complex features of Mu development, and may possibly offer a basis for explaining spontaneous duplications.

The temperate phage Mu-1 is able to integrate at random into the *Escherichia coli* chromosome (7, 11, 30); the integrative recombination takes place at a specific site within the Mu genome, and the prophage is found in either orientation (5, 6, 12, 20, 39). Since Mu prophage and vegetative maps are the same (9, 39) and since Mu prophage and extracellular deoxyribonucleic acid (DNA) are colinear (21), the Mu attachment site is either located close to one end of the phage genome or is formed by association of the two extremities.

Upon infection of a sensitive *recA* host, phage Mu is also able to mediate the integration of a λ-*gal* genome, which is alone unable to integrate into the bacterial chromosome (13). It has been shown that the integrated λ-*gal* may have any circular permutation and may be located at any point in the bacterial chromosome, sandwiched between two complete Mu genomes oriented the same way (33). Similarly, Van de Putte and Gruijthuijsen (34) have found that Mu mediates the integration of an F*lac* episome and enhances chromosome mobilization by F factors. We proposed that such integration events occur as a result of the formation of circular Mu dimers, displaying two attachment sites, one which is able to recombine at any position within the λ-*gal* DNA and the other with any part of the bacterial chromosome (33). This process does not occur upon infection of Mu lysogens, indicating that the Mu functions required to stimulate integration of the λ-*gal* DNA are not expressed under conditions of Mu immunity.

The experiments described here show that λ-*gal* insertion into the chromosome of a Mu lysogen is stimulated under some conditions where at least partial induction of the prophage occurs. In this case, the λ-*gal* DNA again is integrated in any circular permutation and between two Mu genomes having the same orientation; however, the Mu-λ-*gal*-Mu complex is always located at the site of the original Mu prophage.

The stimulation of λ-*gal* integration by different replication-defective Mu prophage mutants was studied. Results indicate that active Mu replication functions are not necessary for the process to occur, and that some mutants of Mu that are unable to promote λ-*gal* integration upon infection are nevertheless able to stimulate λ-*gal* integration upon induction. A model

[1] Present address: U.N.A.M. Instituto de Investigaciones Biomédicas, Apartado Postal 70228, Mexico D.F.
[2] Present address: Institut d'Hygiène et d'Epidémiologie, Ministère de la Santé Publique, Brussels, Belguim.

will be presented that accounts for these observations and may help to explain some more complex features of Mu development.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this work are listed in Table 1.

Phages. The following phages were used: Muc$^+$ (Taylor via Starlinger [30], Muc*ts*61, Muc*ts*62 *Aam1093*, Muc*ts*62 *Bam1066*, Muc*ts*62 *Sam1004* (Howe [20]), Mu*vir3054 Qam3018* (Toussaint et al., to be published), and λ *Nsus7 Nsus53 cI857 r14 gal8* (Rambach and Brachet [27]; will be referred to as λ-*gal*. This phage is unable to integrate or to transduce its *gal* markers into a *recA* strain [27]).

Media. L broth (24) supplemented with 1% maltose (to improve λ-*gal* adsorption) was used for the bacterial cultures; bacterial crosses were performed in L broth + 3×10^{-3} M K_2HPO_4 + 2×10^{-3} M KH_2PO_4.

Bacteria were titrated on tryptone agar, and *gal*$^+$ clones were isolated on 132 minimal medium agar (16) supplemented with 1% galactose, 0.1% Casamino Acids, and biotin (1 μg/ml) when necessary. EMB agar (23) supplemented with 1% galactose was used to analyze the segregation of *gal*$^-$ clones.

Bacterial dilutions were made into 10^{-2} M $MgSO_4$; phage lysates were diluted in SM buffer (36). "A" (15) and tryptone agar media were used to prepare the Mu and λ lysates, respectively.

Phage lysates were prepared by the confluent lysis technique (2). P1 transduction was performed according to Lennox (24).

Superinfection of Mu lysogens with Muvir and λ-gal. Cultures of the Mu lysogens were grown in L broth + 1% maltose, with aeration, to ~2×10^8 bacteria/ml at 37 C (30 C when the prophage was thermoinducible). Samples of 1 ml were infected with Mu*vir* at a multiplicity of infection (MOI) = 3 and/or λ-*gal* at MOI = 10, in the presence of Ca^{2+} (5×10^{-2} M final concentration); after 15 min of adsorption at 37 C (or 30 C), unadsorbed phage were removed by centrifugation (5 min at 5,000 rpm) after 10-fold dilution into 10^{-2} M $MgSO_4$. Pellets were suspended in 1 ml of 10^{-2} M $MgSO_4$ and suitable dilutions were plated at 37 C (or 30 C) on tryptone agar to determine viable counts and on minimal galactose medium to titrate *gal*$^+$ transductants.

Infection of Mucts lysogens with λ-gal. Muc*ts* lysogens were grown in L broth + 1% maltose at 30 C to ~2×10^8 bacteria/ml. One 5-ml portion was diluted twofold into L broth + 1% maltose prewarmed at 30 C, and one 3-ml portion was diluted into 7 ml of the same broth prewarmed at 37 C (42 C when Muc*ts62* X^- mutants were used). Incubation was continued at 30 and 37 C (or 42 C), respectively, to ~2×10^8 bacteria/ml, and 1 ml of each culture was infected with λ-*gal* at MOI = 10. After 15 min of adsorption at the same temperatures, unadsorbed phage were removed and titration was performed as described above.

Characterization of the Mu prophage(s) present in gal$^+$ transductants. *gal*$^+$ transductants derived from strains carrying only a Muc*ts62* S^-, Muc*ts62* B^-, or Muc*ts62* A^- prophage were tested for Mu immunity at 30 C, for phage production at 42 C, and for survival at 42 C. Strains in which Mu immunity was present at 30 C and no phage was produced at 42 C and which died at 42 C were considered to carry only S^-, B^-, and A^- phages, respectively. Muc*ts62* B^- and A^-, inserted in an F′ episome, were transferred by mating into a *sup*$^-$ recipient. Sexductants were subsequently

TABLE 1. *Bacterial strains used*

Strain	Genotype	Source of reference
C600	F$^-$, *thr*$^-$, *leu*$^-$, *thi*$^-$, *lacY*, *supE*	Appleyard (3)
594	F$^-$, *gal1*, *gal2*, Str^r	Campbell (8)
N100	F$^-$, *gal2*, *recA*, Str^r	Meselson via Gottesman and Yarmolinsky (17)
N100 *aspB*::(Muc$^+$)[a]	F$^-$, *aspB*::(Muc$^+$), *gal2*, *recA*, Str^r	Isolated from N100
594 *recA glt*::(Muc*ts62* S^-)	F$^-$, *glt*::(Muc*ts62 Sam1004*), *gal1*, *gal2*, *recA*, Str^r [b]	Isolated from 594
KMBL1617	*Hfr H*, *thi*$^-$, *galK*, *trp*::(− Muc*ts62*)[c]	Wijffelman
RH2608	F$^-$, *glt*::(Muc*ts62 X3998 Sam1004*), *gal1*, *gal2*, *recA*, Str^r	Isolated from 594 *recA glt*::(Muc*ts62* S^-)
RH2609	F$^-$, *glt*::(Muc*ts62 X3999 Sam1004*), *gal1*, *gal2*, *recA*, Str^r	
594 *recA glt*::(Muc*ts62* S^-)/ F*lac*(Muc$^+$)	F$^-$, *glt*::(Muc*ts62 Sam1004*) *lac*$^-$, *gal1*, *gal2*, *recA*, Str^r/F *ts114 lac* (+Muc$^+$)	Isolated from 594 *recA glt*::(Muc*ts62* S^-)
RH2635	F$^-$, Δ(*gal*, *bio*), *malA*, *recA*, Spc^r/F′141 (Muc*ts62 Aam1093*)	Isolated from MR96 and JC1553/F141 (Low and Falkinham [25])
RH2634	F$^-$, Δ(*gal*, *bio*), *malA*, *recA*, Spc^r/F′141 (Muc*ts62 Bam1066*)	
RH2646	F$^-$, Δ(*lac*, *pro*) Y23, Δ(*gal*, λ) X3, *recA*, Str^r/F′ *pro*, *lac*::(Muc*ts62 X5001*)	Isolated from RH1410 (Castellazzi) and HM8564 (Bukhari)
KL16	Hfr, *thi*, *recA*	Low and Wood (26)

[a] :: Indicates that Mu is integrated into the gene which precedes it (Howe and Bade, manuscript in preparation).

[b] The exact nature of the *glt* mutation in this strain has not been determined. The mutation maps between the *argH* and *pyrB* genes.

[c] + Means that Mu is integrated with its immunity gene proximal to the origin of the *E. coli* K-12 map. − Means the other orientation (Howe and Bade, manuscript in preparation).

checked for production of phages growing on *sup*⁻ but not on *sup*⁺ indicators. *gal*⁺ transductants derived from strains carrying both a Muc⁺ and a Muc*ts62* S^- prophage were grown at 30 C to 2 × 10^8 bacteria/ml, and the culture was sterilized by the addition of $CHCl_3$. The lysates were assayed on strains C600 (*sup*⁻) and 594 (*sup*⁺) to screen for *am* phage and at 42 C to screen for *c*⁺ and *cts* phage. *rec*⁺ derivatives of the *gal*⁺ transductants were grown in L broth from a single *gal*⁺ colony, streaked on EMB galactose agar, and incubated at 37 C (30 C where only Muc*ts* prophages were present) to detect *gal*⁻ segregants; *gal*⁻ clones were subsequently tested for the presence or absence of λ and Mu immunity. Following our previous results (33), we considered *gal*⁺ transductants that did not show any segregation to *gal*⁻ as long as they were *recA* and that segregated, in these conditions ~1% λ⁻ *gal*⁻ Mu⁺ clones that produced Mu particles, when they were made *rec*⁺, as carrying a λ-*gal* integrated between two Mu prophages.

Characterization of the λ prophage carried by the gal⁺ transductants. The presence of the whole λ genome in the *gal*⁺ transductants was tested by marker rescue using various λ*i434am* mutants. λ*P*, *R*, *F*, and *K* functions are transactivated after superinfection of a λ lysogen by a heteroimmune λ*i434* (31). Moreover they are catalytic, i.e., required in small amounts (32). For those two reasons, λ*i434P*⁻, *R*⁻, *F*⁻, and *K*⁻ mutants can grow on a noninduced λ lysogen unless those functions required by the superinfectant phage are inactivated in the λ prophage. Transactivation tests were carried out by plating λ*i434Pam*, *Ram*, *Fam*, or *Kam* on lawns of *recA sup*⁺ *gal*⁺ transductants. Inability to complement a superinfecting λ*i434P*⁻ mutant was taken to mean that the integrity of the λ early operon *cII0P* was interrupted due to the Mu-mediated integration; similarly, inability to complement one or more of the late mutants λ*i434R*⁻, λ*i434F*⁻, and λ*i434K*⁻ was taken to mean that λ late operon *S-J* was split after Mu-mediated integration (33).

Conjugation. Donor and recipient strains to be mated were grown in L broth + 3 × 10^{-3} M K_2HPO_4 and 2 × 10^{-3} M KH_2PO_4 to ~2 × 10^8 bacteria/ml at 30 C. One milliliter of the donor was mixed with 1 ml of the recipient for 120 min at 30 C. Dilutions of the mating mixture were plated on minimal selective medium and incubated at 30 C.

RESULTS

Stimulation of λ-gal integration into the chromosome of Mu lysogens by virulent mutants of Mu. Virulent mutants of Mu, i.e., mutants able to grow on Mu lysogens, have been isolated; the nature of the *vir* mutation has not yet been elucidated. *vir* mutants do not lysogenize a sensitive host; upon infection of a Mu lysogen, they induce the resident prophage (Rhodomu, manuscript in preparation). Although Mu*vir* kill the lysogenic host, survivors can be recovered at a high frequency (~50%) when the lysogen is plated immediately after adsorption of the superinfecting Mu*vir*. The survivors retain the Mu prophage and never carry a Mu*vir* (Faelen, unpublished data). We asked whether the Mu functions required to stimulate λ-*gal* integration are expressed during superinfection by Mu*vir*. We compared the frequency of integration of λ*N*⁻*r14gal8* into the chromosome of a Muc⁺ lysogen in the presence and in the absence of co-infecting Mu*vir*. The *gal*⁻ *recA* strain used, N100 *aspB*::(Muc⁺), is most probably a monolysogen, since P1 transduction of the *aspB*⁺ allele in its *rec*⁺ derivative eliminates the Mu prophage, showing the linkage between Mu and the *aspB* mutation. As a control, similar infections were performed in the nonlysogenic strain N100. Mu*vir* did not stimulate integration of λ-*gal* in N100 (Table 2), which was expected since they do not lysogenize and since other mutants of Mu unable to lysogenize (for instance, clear mutants) do not stimulate integration of λ-*gal* in N100 (33); spontaneous integration of λ-*gal* into the chromosome of N100 *aspB*::(Muc⁺) was very inefficient (Table 2), whereas mixed superinfection with λ-*gal* and Mu*vir* gave a 10-fold increase in the number of λ-*gal*⁺ clones.

Analysis of these *gal*⁺ transductants suggests that they carry a Muc⁺-λ-*gal*-Muc⁺ structure, as is the case for λ-*gal* lysogens found upon mixed infection of a sensitive host with λ-*gal* and Muc⁺. Indeed, when *recA* they are stable *gal*⁺, but when made *rec*⁺ (by mating with KL16 *rec*⁺) they segregate ~ 1% λ⁻ *gal*⁻ Muc⁺ clones, suggesting that the λ-*gal* is integrated

TABLE 2. *Integration of λ-gal in sensitive and lysogenic hosts by superinfection with Muc⁺ or Muvir*[a]

Infecting phage	Frequency of *gal*⁺ clones in host	
	N100	N100 *aspB*::(Muc⁺)
None	<3.1 × 10^{-8}	<2.5 × 10^{-8}
Muc⁺	<4 × 10^{-8}	<3 × 10^{-8}
Mu*vir*[b]	<5 × 10^{-8}	<7 × 10^{-8}
λ-*gal*[c]	<4.2 × 10^{-7}	2.8 × 10^{-7}
λ-*gal* + Muc⁺	2 × 10^{-4}	3 × 10^{-7}
λ-*gal* + Mu*vir*	<3.4 × 10^{-8}	2.7 × 10^{-6}

[a] N100 and N100 *aspB*::(Muc⁺) were grown to mid-log phase at 37 C and infected with Mu (MOI = 3) and/or λ-*gal* (MOI = 10). After 15 min of adsorption at 37 C, the mixtures were diluted and plated upon tryptone agar and minimal Casamino Acids galactose medium. The frequency of *gal*⁺ transductants is given by the ratio (viable counts on minimal galactose agar)/(viable counts on tryptone agar).

[b] Mu*vir* is Mu*vir*3054 *Qam*3018.

[c] λ-*gal* is λ *Nsus7 Nsus53 cI857 r14 gal8*; this phage is unable to integrate by itself in a *recA sup*⁺ host (27).

between two Muc$^+$ prophages in the same orientation. The λ-*gal* itself is most probably integrated in any circular permutation since different sets of λ genes are inactivated in different *gal*$^+$ transductants (see Material and Methods for details). P1 transduction of the *aspB*$^+$ allele in the *rec*$^+$ *gal*$^+$ transductants systematically results in the loss of both the λ-*gal* and Muc$^+$ prophages, suggesting that the Mu-λ-*gal*-Mu complex is always located at the *aspB* locus, the site previously occupied by the original Muc$^+$. This is different from what was found in λ-*gal* lysogens isolated upon mixed infection of N100, where the Mu-λ-*gal* Mu complex was found anywhere in the bacterial chromosome (33).

It seems unlikely that, in the case described here, the prophage located in the *aspB* gene excises, duplicates, and, according to the model we have described (33), forms a dimer which in this case would always reintegrate with the λ-*gal* precisely at the site occupied by the original Mu prophage. Nevertheless, to rule out this possibility, strain N100 *aspB*::(Muc$^+$) was superinfected with λ-*gal*, Mu*vir*, and Muc*ts61* at 30 C to investigate whether one or two Muc*ts61* genomes can be integrated into its *aspB* gene. A total of 330 *gal*$^+$ transductants were isolated and analyzed. Since none exclusively produced Muc*ts61*, the Muc$^+$ prophage cannot simply be replaced by a Muc*ts61*-λ-*gal*-Muc*ts61* complex. Of these clones, 320 produced only Muc$^+$ phages; 10 others were found to release both Muc$^+$ and Muc*ts61* particles. Segregation and P1 transduction data from *rec*$^+$ derivatives of these 10 clones showed that 6 carried a λ-*gal* integrated between two Muc$^+$ prophages at the site of the *aspB* gene and also a Muc*ts61* prophage elsewhere in the host chromosome. Three other clones carried a Muc$^+$ at the *aspB* site and a Muc*ts61*-λ-*gal*-Muc*ts61* structure outside *aspB*. The last isolate seemed to have its λ-*gal* sandwiched between a Muc$^+$ and a Muc*ts* in the *aspB* gene. However, this particular lysogen probably resulted from multiple events, since its Muc*ts* prophage differs from the Muc*ts61* used in the experiment in that it is unable to lysogenize even at 30 C and that *gal*$^-$ segregants containing only this Muc*ts* are inviable at 30 C. These results indicate that the original Muc$^+$ prophage is always maintained in the *aspB* gene and that in the majority of the cases integration of the λ-*gal* at the *aspB* locus cannot involve an interaction between a superinfecting Muc*ts*61 DNA and the Muc$^+$ prophage.

Another experiment gave similar conclusions. The merodiploid strain 594 *recA glt*::(Muc*ts62* S^-)/F*lac* (Muc$^+$) was superinfected with λ-*gal* and Mu*vir* at 30 C, and *gal*$^+$ transductants were selected at 30 C and analyzed (Table 3). The majority carried the λ-*gal* inserted between two Muc$^+$ genomes in the episome (77 of 120 colonies) or between two Muc*ts62* S^- in the *glt* gene (42 of 120 colonies). One strain was found to have its λ-*gal* flanked by two Muc$^+$ at a site in the bacterial chromosome outside the *glt* locus, but none of the transductants carried the λ-*gal* sandwiched between a Muc$^+$ and a Muc*ts*62 S^- either in the bacterial chromosome or in the episome. This strongly suggests that the two Mu genomes flanking the λ-*gal* DNA in the *gal*$^+$ transductants derive from the same Mu prophage.

Stimulation of λ-gal integration in Muc*ts* lysogens grown at 37 C. Strain KMBL1617, monolysogenic for a thermoinducible Muc*ts62*, is killed by incubation at 42 C; nevertheless, survivors are recovered at a frequency of $\sim 10^{-5}$. The same lysogen survives at 37 C at a frequency of 0.2 to 0.5. The yields of phage from this lysogen at 30, 37, and 42 C are, respectively, 5×10^{-6}, 10^{-2}, and 50 phage per bacterium. Van de Putte (personal communication) found that Muc*ts62* monolysogens grown at 37 C often generate polylysogenic derivatives. Polylysogenization seems to indicate that both replication and integration functions of the prophage are expressed under those conditions. Expression of these functions could in theory also stimulate Mu-promoted λ-*gal* integration. We tested this possibility by comparing the frequency of integration at 30 C of λ *N*$^-$ *r14 gal8* into the chromosome of strain 594 *recA glt*::(Muc*ts62 Sam 1004*) to the frequency at 37 C. The S^- mutation prevented the Muc*ts62*

TABLE 3. *Analysis of gal*$^+$ *transductants obtained after infection of the merodiploid strain 594 recA glt*::(*Muc*ts62 S^-)/*Flac*(*Muc*$^+$) *with Muvir and* λ-*gal*

No. of clones of each type	Phage present in cells cured of the episome[a]	Phage linked to the episome[b]	Phage linked to the *glt* gene[c]
77	Muc*ts*	Muc$^+$, λ-*gal*	Muc*ts*
42	Muc*ts*, λ-*gal*	Muc$^+$	Muc*ts*, λ-*gal*
1	Muc*ts*, Muc$^+$, λ-*gal*	Muc$^+$	Muc*ts*

[a] Cells cured of the episome were obtained among survivors of treatment with the male-specific phage f_2.

[b] Linkage between the prophage and the episome was determined by testing co-transference of the prophage and an episomal marker in conjugation with a *gal*$^-$ *lac*$^-$ recipient.

[c] Phages genomes linked to the *glt* region were identified by transducing the *glt*$^+$ allele with phage P1 into *rec*$^+$ derivatives of the cured cells and examining the transductants for prophage loss.

from complete maturation, minimizing phage production and subsequent readsorption of free phage during growth at 37 C. Integration of λ-*gal* into the host chromosome was very inefficient at 30 C (Table 4); however, *gal*$^+$ transductants were recovered at an elevated frequency when the experiment was performed at 37 C. Thirty of such *gal*$^+$ clones produced at 37 C were purified and further analyzed. They were all found to be killed by incubation at 42 C; none, however, produced mature phage. This indicates that their Mu*cts62* prophage(s) still carried the *S*$^-$ mutation. Marker rescue and transactivation data on the λ-*gal* prophages suggest that the integrative recombination occurs randomly within the λ-*gal* genome.

In most cases (27 of 30), P1 transduction of the *glt*$^+$ allele in the *rec*$^+$ derivatives of those *gal*$^+$ transductants eliminated the Mu and λ-*gal* prophages; in the three remaining strains, only λ-*gal* was eliminated by that process. Moreover, although all *recA* transductants were stable *gal*$^+$, when made *rec*$^+$ they segregated ~1% λ$^-$ *gal*$^-$ Mu*cts62* *S*$^-$ clones. These results suggest that the 30 transductants analyzed carried a Mu*cts62* *S*$^-$-λ-*gal*-Mu*cts62* *S*$^-$ in the *glt* gene, three of them having an additional Mu*cts62* *S*$^-$ located outside *glt*.

The orientation of the Mu*cts62* *S*$^-$ prophage(s) in the *gal*$^-$ parent and in 20 *gal*$^+$ transductants was determined according to the method of Zeldis et al. (40). An F*lac* (+ Muc$^+$) episome was introduced into *rec*$^+$ derivatives of the strains to be tested. The mobilization by the F*lac* (+ Muc$^+$) episome of genes *argH* and *pyrB*, which surround the Mu*cts62* *S*$^-$ prophage(s), was measured by mating these *rec*$^+$/F*lac* (+ Muc$^+$) derivatives with F$^-$*argH* and F$^-$*pyrB* recipients. Since mobilization occurs by recombination between the Mu prophages carried by the episome and the host chromosome, the ratio of *argH*$^+$ to *pyrB*$^+$ recombinants depends upon the orientation of the Mu prophage in the chromosome. All the F′ strains tested mobilized the *argH* gene at a higher frequency than the *pyrB* gene (results not shown) indicating that in all strains the Mu*cts62* *S*$^-$ prophage(s) has the same orientation. This is again consistent with the idea that the original prophage does not excise and reintegrate in the *glt* gene; indeed, if this were the case, one would expect to find some reintegrated prophages having the opposite orientation.

Stimulation of λ-gal integration by Mu prophages carrying A$^-$, B$^-$, or X$^-$ mutations. Mu mutants affected in gene *A* or *B* (1) have been shown to be unable to stimulate integration of a λ-*gal* (33) or F*lac* (34) upon infection.

TABLE 4. *Integration of λ-gal into the chromosome of strain 594 recA glt::(Mucts62 S$^-$) grown at 30 and 37 C*[a]

Infecting phage	Frequency of *gal*$^+$ clones in cultures grown at:		Ratio 37 C/30 C
	30 C	37 C	
None	$<3.6 \times 10^{-8}$	$<4.4 \times 10^{-8}$	
λ-*gal*	3×10^{-7}	7.7×10^{-5}	258

[a] Cultures of 594 *recA glt*::(Mu*cts62 Sam1004*) were grown at 30 and 37 C, infected with λ-*gal* (MOI = 10), and plated on tryptone agar or minimal Casamino Acids galactose agar. The frequency of *gal*$^+$ transductants is given by the ratio (viable counts on minimal galactose agar)/(viable counts on tryptone agar).

The products of these genes are also directly or indirectly necessary for Mu replication since phage mutants in these genes are unable to replicate their DNA upon infection (37) or induction (Faelen, unpublished data). Moreover, they are unable to synthesize late messenger ribonucleic acid (RNA) (37). We therefore compared the frequency of λ-*gal* integration into the chromosome of a *gal*$^-$ *recA* (Mu*cts62* *A*$^-$) strain (RH2635) and a *gal*$^-$ *recA* (Mu*cts62* *B*$^-$) strain (RH2634) when grown at 30 and 37 C. The Mucts62 A$^-$ prophage could not stimulate the integration of the λ-*gal* even at 37 C, whereas the Mu*cts62* *B*$^-$ retained the ability to do so (Table 5). Such λ$^+$-*gal*$^+$ transductants carry only Mu phage, suggesting that the *B* gene product is unnecessary for λ-*gal* insertion and consequently that Mu replication is not a prerequisite for λ-*gal* integration. *X*$^-$ mutants of Mu (Bukhari, submitted for publication) are also unable to replicate and to synthesize late RNA (Bukhari, personal communication). Moreover, unlike Mu*cts62* *A*$^-$ and Mu*cts62* *B*$^-$ lysogens, the Mu*cts62* *X*$^-$ lysogens are not killed by incubation at 42 C. We examined λ-*gal* integration into the chromosome of *recA* *gal*$^-$ (Mu*cts62* *X*$^-$) strains. Three strains carrying different *X*$^-$ mutant prophages were grown at 30 and 42 C and infected with λ *N*$^-$ *r14* *gal8*. All strains integrated λ-*gal* very poorly at 30 C; however, incubation at 42 C stimulated λ-*gal* insertion by a factor of over 100 (Table 6). Ten *gal*$^+$ transductants derived from RH2646, which carries the Mu*cts62* *X*$^-$ prophage upon an F*lac pro* episome, were further analyzed. They did not produce mature phage and did not die when incubated at 42 C. Loss of the F*lac pro* episome was associated in all cases with simultaneous loss of the Mu*cts62* *X*$^-$ and λ-*gal* prophages, and all prophages were transferred

TABLE 5. *Integration of λ-gal into the chromosome of sup⁺ recA gal⁻ (Mucts62 Aam1093) and sup⁺ recA gal⁻ (Mucts62 Bam1066) strains grown at 30 and 37 C*[a]

Strain	Infecting phage	Frequency of *gal*⁺ clones in cultures grown at:		Ratio 37 C/30 C
		30 C	37 C	
RH2635	None	$<2 \times 10^{-8}$	$<2.2 \times 10^{-8}$	
sup⁺recA gal⁻ (Mucts62 Aam 1093)	λ-*gal*	2.7×10^{-7}	5×10^{-7}	1.8
RH2634	None	$<2.7 \times 10^{-8}$	$<2.5 \times 10^{-8}$	
sup⁺recA gal⁻ (Mucts62 Bam 1066)	λ-*gal*	2.1×10^{-7}	2.2×10^{-5}	104

[a] Cultures of RH2635 and RH2634 grown at 30 and 37 C were infected with λ-*gal* (MOI = 10) and plated upon tryptone agar and minimal Casamino Acids galactose medium. Frequency of *gal*⁺ transductants is given by the ratio (viable counts on minimal galactose agar)/(viable counts on tryptone agar).

TABLE 6. *Integration of λ-gal into the chromosome of various sup⁺ recA gal⁻ (Mucts62X⁻) strains grown at 30 and 42 C*[a]

Strain	Infecting phage	Frequency of *gal*⁺ clones in cultures grown at:		Ratio 42 C/30 C
		30 C	42 C	
RH2646	None	$<3 \times 10^{-8}$	$<4 \times 10^{-8}$	
	λ-*gal*	3×10^{-7}	1.2×10^{-4}	400
RH2608	None	$<2 \times 10^{-8}$	$<3.6 \times 10^{-8}$	
	λ-*gal*	4.4×10^{-7}	3.3×10^{-4}	750
RH2609	None	$<2.5 \times 10^{-8}$	$<3 \times 10^{-8}$	
	λ-*gal*	6.1×10^{-7}	2×10^{-4}	330

[a] Cultures of RH2646, RH2608, and RH2609 were grown at 30 and 42 C, infected with λ-*gal* (MOI = 10), and plated on minimal Casamino Acids galactose medium and tryptone agar. Frequency of *gal*⁺ transductants is given by the ratio (viable counts on minimal galactose agar)/(viable counts on tryptone agar).

simultaneously by the episome to an F⁻ *gal*⁻ recipient. Where the recipient was *rec*⁺ *gal*⁻, the *gal*⁺ sexductants efficiently segregated λ-*gal*⁻ clones that still retained an F*pro lac*::(Mu*cts62* X^-) episome. The λ-*gal* genome is thus most probably integrated in the episome between two Mu*cts62* X^- prophages.

DISCUSSION

The experiments described here show that a λ-*gal* genome, which is by itself unable to integrate into the chromosome of a λ-sensitive host, can be inserted into the chromosome of a Mu lysogen where the prophage has been at least partially induced. The *gal*⁺ transductants obtained by such means carry the λ-*gal* in any circular permutation, flanked by two whole Mu prophages having the same orientation and the same genotype as the original Mu prophage. Moreover, the Mu-λ-*gal*-Mu structure is in the large majority of the cases located at the site previously occupied by the single Mu.

There are two striking differences between these results and what was found earlier after mixed infection of a nonlysogenic host with the same λ-*gal* and Muc⁺. In that case, the Mu-λ-*gal*-Mu structure may be integrated at any site within the bacterial chromosome, and Muc⁺-λ-*gal*-Mu*cts* structures have been found after simultaneous infection with Muc⁺, λ-*gal*, and Mu*cts* (33). In the case presented here, it seems that the prophage directly participates in the integration of the λ-*gal* genome. The parental strains N100 *aspB*::(Muc⁺) and 594 *recA glt*::(Mu*cts62* S^-) will be considered as monolysogens, since P1 transduction of the *aspB*⁺ and *glt*⁺ allele, respectively, eliminates the Mu prophage.

This does not eliminate the possibility that those two strains carry a Mu tandem in *aspB* or *glt*; such a tandem could carry a Mu attachment site at the junction of the two prophages that could be used for integrating the λ-*gal* DNA. However, we will not consider that possibility; indeed, it would lead us to consider that all the *recA* lysogens used in this work carry a Mu tandem, and this would contradict the fact that none of the strains considered as monolysogens by genetic criteria whose prophage was vizualized by electron microscopy did show a Mu tandem. The *gal*⁺ transductants isolated from N100*aspB*::(Muc⁺) and 594 *recA glt*::(Mu*cts62* S^-) are most probably at least dilysogens, as shown by their pattern of segregation to *gal*⁻ clones. However, duplication of the Mu prophage does not seem to require Mu replication machinery, since strains carrying a Mu*cts62* prophage mutated in gene *B* or *X* (the prophage thus being unable to replicate) can still promote λ-*gal* integration at about the same frequency. We would like to propose a

model in which λ-*gal* insertion occurs at the site of the Mu prophage when the bacterial replication fork has passed through that region, providing two copies of the Mu prophage (Fig. 1). There is good evidence to suggest that phage Mu codes for an endonuclease that can catalyze the formation of a specific double-stranded cut at one extremity of the Mu prophage (29; Schröder, submitted for publication). This enzyme could possibly cut at the end of only one of the two parallel Mu genomes and, once exposed, this extremity would be able to react with the opposite extremity of the second Mu prophage, generating between them an active Mu attachment site (Fig. 1[3]). This site would be available for a recombination with any sequence of a circular λ-*gal* DNA (Fig. 1[4]). If subsequently the bacterial chromosome is correctly repaired, a viable bacterium will be created that carries the λ-*gal* prophage in any circular permutation, sandwiched between two Mu prophages identical to the original Mu; the whole Mu-λ-*gal*-Mu structure is at the same location in the chromosome as the original prophage (Fig. 1[6]). The model proposed here is not solely applicable to Mu genetics; the way in which the prophage dimerizes is an example of duplication that might pertinently be applied to the spontaneous duplication of bacterial regions (4, 14, 18, 20) if some bacterial sequences can be handled by bacterial enzyme(s) as are the Mu extremities by the Mu integration enzyme(s). The structure shown in Fig. 1(3) and (4), where one Mu*A* extremity interacts in some way with the opposite Mu*B* extremity, which is covalently linked to a bacterial sequence, could be a Mu attachment site. It is known that one end of Mu vegetative DNA is a random bacterial sequence (Bade, Daniell, and Abelson, personal communication), called the variable end, that represents ~3% of the total length of Mu DNA (10). The variable end is most probably lost when Mu integrates into the bacterial chromosome (22). One could imagine that integration of Mu DNA proceeds by interaction between the Mu*A* site located at the fixed extremity of Mu vegetative DNA and a Mu*B* site located at the other extremity, beyond the variable end, while Mu*B* is still linked to that bacterial sequence, as it is the case in Fig. 1(3) and (4); this structure could be recognized by the Mu integration enzyme(s), those being able to either integrate Mu DNA and release the variable end (in the case of Mu integration) or to integrate the λ-*gal* and release the *c* bacterial sequence (in the case of Mu-mediated λ-*gal* integration).

Other conclusions regarding Mu integration

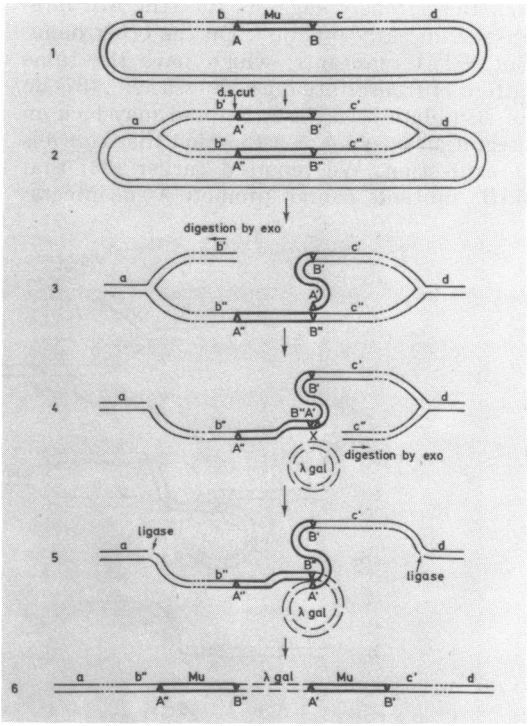

FIG. 1. (*1*) *The Mu prophage is inserted between the bacterial sequences b and c.* (*2*) *The bacterial replication fork has passed through the b-Mu-c region of the chromosome, providing two parallel copies of that region.* (*3*) *A specific Mu endonuclease makes a double stranded cut at one extremity of the Mu prophage, liberating a MuA' sequence, which interacts with the opposite end of the second prophage, MuB", generating a Mu attachment site. The b' bacterial sequence is then degraded by bacterial exonuclease(s).* (*4*) *The A'B" site is able to recombine with any sequence of a circular λ-gal DNA, this process requiring Mu integration enzyme(s). The c" bacterial sequence is then degraded by bacterial exonuclease(s).* (*5*) *The exposed branches at either side of the Mu dimer have been degraded, and the chromosome has been resealed by the bacterial ligase.* (*6*) *The bacterial chromosome now contains a λ-gal prophage, in any circular permutation, sandwiched between two identical Mu prophages that have the same orientation.*

enzymes and Mu regulation can be made from the experiments presented here. Strains lysogenic for Mu*cts62* B^- or Mu*cts62* X^- are able to stimulate integration of λ-*gal*, the resulting *gal*$^+$ transductants carrying a λ-*gal* flanked by two Mu*cts62* B^- or Mu*cts62* X^-, respectively. B^- and X^- mutants of phage Mu are thus able to provide integration enzymes. Since both mutants synthesize only early messenger RNA

(37), this strongly suggests that the Mu integrase is an early function. On the other hand, Muc*ts62* A^- mutants, which have the same pattern of transcription as Muc*ts62* B^-, (37) do not stimulate λ-*gal* insertion and may lack an integration enzyme or a function that controls its expression. We reported earlier (33) that MuB^- mutants cannot promote λ-*gal* integration as revealed by mixed infection of a sensitive *recA gal*$^-$ strain with λ-*gal* and Muc*tsB*$^-$ at 30 C. The present results show that this is most probably not due to an inability to synthesize active integration enzyme(s) but rather due to some other defect, such as an inability to replicate. Muc*ts62* X^- lysogens, which are isolated as survivors at 42 C of a Muc*ts62* lysogen,

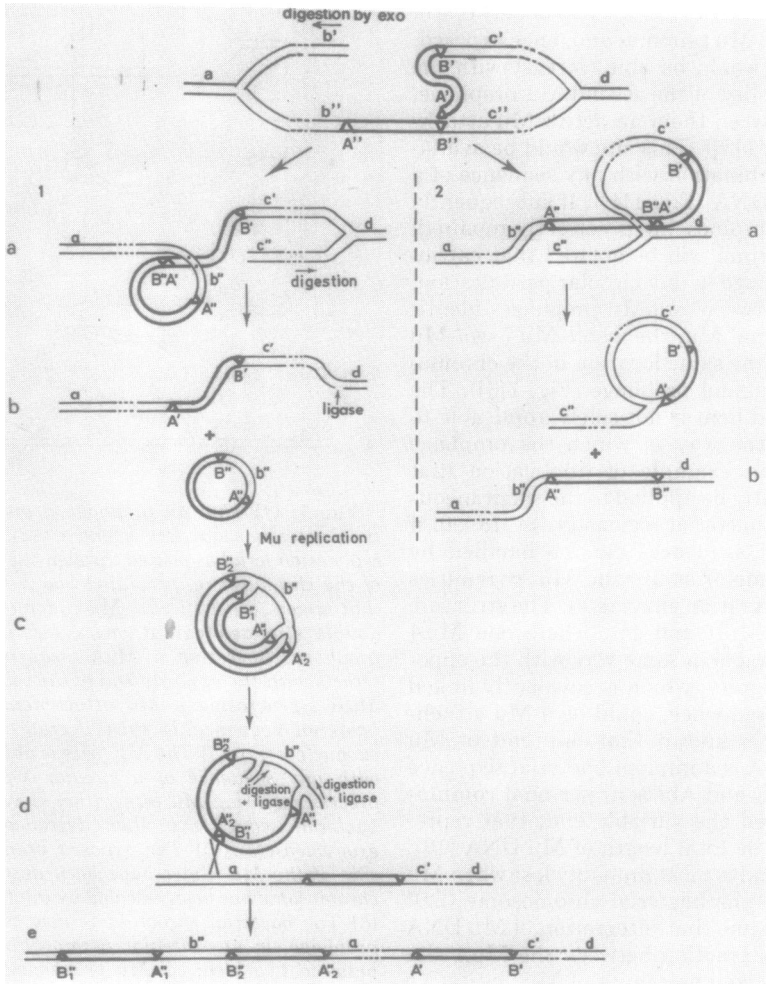

FIG. 2. *Following steps 1, 2, and 3 of Fig. 1, the Mu site A'B'' has been formed and is available for recombination with the adjacent bacterial DNA, either in the duplicated region (1) or beyond the replication fork (2). Recombination within the duplicated region will lead to the formation of a covalently closed circle containing one Mu DNA and the b'' bacterial sequence. The host chromosome, still containing one Mu prophage, could possibly be repaired (1b). If Mu replication is bidirectional, the mixed circle Mu-b'' may generate the structure shown in 1c, containing two copies of the Mu DNA. After a double-stranded cut at one end of one of the Mu prophages, as shown in Fig. 1(3), a Mu attachment site A''$_2$ B''$_1$ may be generated and may then itself recombine with any sequence of the bacterial chromosome (1d). The structure generated (1e) will be comprised of one Mu prophage, which now carries the bacterial sequence "a" at its A extremity (whereas the original Mu prophage had the b sequence at its A extremity), and where the b'' sequence has been transposed to beside the bacterial sequence a. In 2a, the Mu site A'B'' recombines with the bacterial chromosomes outside the duplicated region; this generates a tailed circle, containing one Mu genome and the bacterial sequences c' and c'', which has the structure equivalent to that of a rolling circle (2b).*

integrate λ-*gal* when further grown at 42 C but not after growth at 30 C for several generations. This strongly suggests that Mu immunity is restored in Mu*cts62* X^- lysogens grown at low temperature.

A further look at the model presented in Fig. 1 led us to discover that, by using the Mu site implicated in the integration of the λ-*gal* genome (Fig. 1[4]) to recombine with neighboring bacterial DNA, one finds several interesting DNA structures. If such recombination takes place within the duplicated region of the host chromosome, a circle containing one Mu DNA and a piece of bacterial DNA of variable size (mixed circle) will be generated (Fig. 2[1]). Should the recombination occur beyond the replication fork, this will generate a tailed circle containing one Mu and variable amounts of host DNA, including the replication fork; this circle thus has the structure of a rolling circle (Fig. 2[2]). Both mixed and tailed circles of variable size that might be similar to the circles described here have been found in the fast-sedimenting fraction of Mu DNA isolated after prophage induction or phage infection (28, 35; Schröder, personal communication). Duplication of the Mu in a mixed circle could possibly result in the reintegration of that structure into the bacterial chromosome (Fig. 2[1]), in which case the bacterial sequence carried by the circle will be moved from its original location and incorporated elsewhere into the host chromosome (translocation) between two Mu prophages (Fig. 2[1d]. It has been found that after induction of a Mu*cts62* lysogen, reintegration of the phage elsewhere in the bacterial chromosome or in an F′ episome occurs quite frequently (Schröder and Bukhari, personal communication). On the other hand, we found that translocation of the *pyrF* locus into an F*lac* episome occurs in a *recA* strain grown at 37 C that carries one Mu*cts62* prophage integrated into the nearby *trp* operon. The F*lac pyrF* episomes obtained in this way also carry at least one Mu*cts62* prophage and segregate F*lac* (Mu*cts62*) episomes at a frequency of ~1% if transferred to a *rec*$^+$ background. This suggests that the translocated *pyrF* segment may be flanked by two Mu*cts62* prophages in the same orientation (Faelen and Toussaint, in preparation).

Partially duplicated mixed circles could, on the other hand, reintegrate into another circle of similar structure, generating a larger mixed circle made up of the two bacterial sequences from the parental circles and three Mu prophages, of which two will always have the same orientation since they derive from a single original Mu prophage; the third prophage may, however, have the same or the opposite orientation. This is consistent with the findings by Schröder et al. (28) that early circles contain less Mu DNA than circles isolated late in infection.

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LITERATURE CITED

1. Abelson, J., W. Boram, A. I. Bukhari, M. Faelen, M. Howe, M. Metlay, A. L. Taylor, A. Toussaint, P. Van de Putte, G. C. Westmaas, and C. A. Wyffelman. 1973. Summary of the genetic mapping of prophage Mu. Virology **54**:90-92.
2. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, New York.
3. Appleyard, R. K. 1954. Segregation of lambda lysogenicity during bacterial recombination in *E. coli* K12. Genetics **39**:429-439.
4. Beeftinck, F., R. Cunin, and N. Glansdorff. 1974. Arginine gene duplication in recombination deficient and proficient strains of *E. coli* K 12. Mol. Gen. Genet. **132**:241-253.
5. Boram, W., and J. Abelson. 1973. Bacteriophage Mu integration: on the orientation of the prophage. Virology **54**:102-108.
6. Bukhari, A. I., and M. Metlay. 1973. Genetic mapping of prophage Mu. Virology **54**:109-116.
7. Bukhari, A. I., and D. Zipser. 1972. Random insertion of Mu-1 DNA within a single gene. Nature (London) New Biol. **236**:240-243.
8. Campbell, A. 1961. Sensitive mutants of bacteriophage λ. Virology **14**:22-32.
9. Couturier, M., and F. Van Vliet. 1974. Vegetative recombination in bacteriophage Mu-1. Virology **60**:1-8.
10. Daniell, E., J. Abelson, J. S. Kim, and N. Davison. 1973. Heteroduplex structures of bacteriophage Mu DNA. Virology **51**:237-239.
11. Daniell, E., R. Roberts, and J. Abelson. 1972. Mutations in the lactose operon caused by bacteriophage Mu. J. Mol. Biol. **69**:1-8.
12. Faelen, M., and A. Toussaint. 1973. Isolation of conditional defective mutants of temperate phage Mu-1 and deletion mapping of the Mu prophage. Virology **54**:117-124.
13. Faelen, M., A. Toussaint, and M. Couturier. 1971. Mu-1 promoted integration of a λ-*gal* phage in the chromosome of *E. coli*. Mol. Gen. Genet. **113**:367-370.
14. Folk, W. R., and P. Berg. 1971. Duplication of the structural gene for glycyl-transfer RNA synthetase in *E. coli*. J. Mol. Biol. **58**:595-610.
15. Fry, B. A. 1959. Conditions for the infection of *E. coli* with lambda phage and for establishment of lysogeny. J. Gen. Microbiol. **21**:676-689.
16. Glansdorff, N. 1965. Topography of cotransducible arginine mutations in *E. coli* K 12. Genetics **51**:167-179.
17. Gottesman, M., and M. Yarmolinsky. 1968. The integration and excision of bacteriophage λ genome. Cold Spring Harbor Symp. Quant. Biol. **33**:735-739.
18. Hill, C. W., J. Fouds, L. Soll, and P. Berg. 1969. Instability of a missense suppressor resulting from a duplication of genetic material. J. Mol. Biol. **39**:563-581.

19. Horiuchi, T., S. Horiuchi, and A. Novick. 1963. The genetic basis of hypersynthesis of β galactosidase. Genetics **48**:157-169.
20. Howe, M. 1973. Prophage deletion mapping of bacteriophage Mu-1. Virology **54**:93-101.
21. Hsu, M. T., and N. Davison. 1972. Structure of inserted bacteriophage Mu-1 DNA and physical mapping of bacterial genes by Mu-1 DNA insertion. Proc. Nat. Acad. Sci. U.S.A. **69**:2823-2827.
22. Hsu, M. T., and N. Davison. 1974. Electron microscope heteroduplex study of the heterogeneity of Mu phage and prophage DNA. Virology **58**:229-239.
23. Lederberg, J. 1947. Gene recombination and linked segregation in *E. coli*. Genetics **32**:505-525.
24. Lennox, E. S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190-206.
25. Low, R. B., and J. Falkinham. 1972. *In* A. I. Laskin and H. Lechevalier (ed.), Handbook of microbiology. Chemical Rubber Co., Cleveland, Ohio.
26. Low, R. B., and T. H. Wood. 1965. A quick and efficient method for interruption in bacterial conjugation. Genet. Res. **6**:300-303.
27. Rambach, A., and P. Brachet. 1971. Selection de mutants du bacteriophage λ incapables de se répliquer. C. R. Acad. Sci. **272**:149.
28. Schröder, W., E. G. Bade, and H. Delius. 1974. Participation of *E. coli* DNA in the replication of temperate bacteriophage Mu-1. Virology **60**:534-542.
29. Schröder, W., and P. Van de Putte. 1974. Genetic study of prophage excision with a temperature inducible mutant of Mu-1. Mol. Gen. Genet. **130**:99-104.
30. Taylor, A. L. 1963. Bacteriophage induced mutations in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. **50**:1043-1051.
31. Thomas, R. 1966. Control of development in temperate bacteriophages. I. Induction of prophage functions following heteroimmune superinfection, J. Mol. Biol. **22**:79-95.
32. Thomas, R., C. Leurs, C. Dambly, D. Parmentier, L. Lambert, P. Brachet, N. Lefebvre, S. Mousset, J. Porcheret, J. Szpirer, and D. Wauters. 1967. Isolation and characterization of new *sus* (am) mutants of bacteriophage λ. Mutat. Res. **4**:735-741.
33. Toussaint, A., and M. Faelen. 1973. Connecting two unrelated DNA sequences with a Mu dimer. Nature (London) New Biol. **242**:1-4.
34. Van de Putte, P., and M. Gruijthuijsen. 1972. Chromosome mobilization and integration of F factors in the chromosome of *recA* strains of *E. coli* under the influence of bacteriophage Mu-1. Mol. Gen. Genet. **118**:173-183.
35. Waggoner, B. T., N. S. Gonzales, and A. L. Taylor. 1974. Isolation of heterogeneous circular DNA from induced lysogens of bacteriophage Mu-1. Proc. Nat. Acad. Sci. U.S.A. **71**:1255-1259.
36. Weigle, J., M. Meselson, and K. Paigen. 1959. Density alterations associated with transducing ability in the bacteriophage lambda. J. Mol. Biol. **1**:379-386.
37. Wijffelman, C., M. Gassler, W. F. Stevens, and P. Van de Putte. 1974. On the control of transcription of bacteriophage Mu. Mol. Gen. Genet. **131**:85-96.
38. Wijffelman, C., G. C. Westmaas, and P. Van de Putte. 1972. Vegetative recombination of bacteriophage Mu-1. Mol. Gen. Genet. **116**:40-46.
39. Wijffelman, C., G. C. Westmaas, and P. Van de Putte. 1973. Similarity of vegetative map and prophage map of bacteriophage Mu-1. Virology **54**:125-134.
40. Zeldis, J. B., A. I. Bukhari, and D. Zipser. 1973. Orientation of prophage Mu. Virology **55**:289-294.