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# State of prophage Mu DNA upon induction

**(bacteriophage Mu/bacteriophage λ/DNA insertion/DNA excision/transposable elements)**

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ABSTRACT **We have compared the process of prophage λ induction with that of prophage Mu. According to the Campbell model, rescue of λ DNA from the host DNA involves reversal of λ integration such that the prophage DNA is excised from the host chromosome. We have monitored this event by locating the prophage DNA with a technique in which DNA of the lysogenic cells is cleaved with a restriction endonuclease and fractionated in agarose gels. The DNA fragments are denatured in gels, transferred to a nitrocellulose paper, and hybridized with $^{32}$P-labeled mature phage DNA. The fragments containing prophage DNA become visible after autoradiography. Upon prophage λ induction, the phage–host junction fragments disappear and the fragment containing the λ *att* site appears. No such excision is seen in prophage Mu. The Mu–host junction fragments remain intact well into the lytic cycle, when Mu DNA has undergone many rounds of replication and apparently many copies of Mu DNA have been integrated into the host DNA. Therefore, we postulate that Mu DNA replicates *in situ* and that the replication generates a form of Mu DNA active in the integrative recombination between Mu DNA and host DNA. This type of mechanism may be common to many transposable elements.**

Assimilation of one DNA molecule into another is a fundamental biological phenomenon spanning the whole range of prokaryotic and eukaryotic organisms. The insertion problem has been studied in its most clear-cut form in viral systems. The classic mode of integration of viral genomes involves recombination between the host DNA and a circular form of the inserting viral DNA. Specific sequences in the inserting circular genomes and in the host DNA are recognized for recombination, so that the process culminates in complete linear insertion of the circles (1). Rescue of the inserted DNA from the host is visualized as physical excision of the DNA in a manner that is a reversal of the insertion process. The question of whether or not the temperate bacteriophage Mu conforms to this classic mode of integration and excision has been the focus of the current work on Mu (2). This question has arisen because Mu is strikingly different from other temperate viruses of bacteria in several features.

Unlike other temperate phages, Mu inserts its DNA at randomly distributed sites on the genome of its host bacterium *Escherichia coli* (3, 4). As extracted from mature phage particles, Mu DNA, is a linear DNA duplex of 37–38 kilobases and has at its ends host DNA that differs in size and sequence from molecule to molecule (5, 6). Thus, Mu does not have terminally cohesive or repetitious sequences and lacks any obvious means of fusing its ends to form circular DNA molecules. The terminal host sequences are randomized during Mu growth, because Mu lysates grown from a single plaque still contain particles with different host sequences. Yet, a form of Mu DNA free of host DNA has remained undetected.

In its continuous association with host DNA, Mu resembles another class of insertion elements, referred to as the transposable elements. The transposable elements are specific stretches of DNA that can be translocated from one position to another in host DNA (7). Mu undergoes multiple rounds of transposition during its growth, far exceeding the transposition frequency of the *bona fide* transposable elements. When a Mu lysogen, carrying a single Mu prophage at a given site on the host chromosome, is induced, many copies of Mu DNA are rapidly integrated at different sites as the replication of Mu DNA proceeds.

We have sought to determine whether induction of a Mu prophage, with subsequent replication and transposition of Mu DNA, involves excision of the prophage DNA from the original site. To do this, we have examined the fate of prophage Mu DNA, and also of prophage λ DNA, *in situ* in the host chromosome after induction. This paper presents evidence that, unlike λ, prophage Mu DNA persists at its original site after induction.

## MATERIALS AND METHODS

**Bacterial Strains.** The *E. coli* strains were all derivatives of *E. coli* K-12. The basic bacteriophages in the lysogenic cells were either Mu *cts*62, a temperature-inducible mutant of bacteriophage Mu carrying a mutation in the immunity gene *c* (8), or λ *c*I857S7, a temperature-inducible derivative of bacteriophage λ. The Mu *cts*62 lysogens were: BU563 (Mu *cts*62 located in one of the *pro* genes), BU568 (Mu *cts*62 located at the *thr* locus), BU575 (Mu *cts*62 located at the *trp* locus), BU8220 (Mu *cts*62 located in the *lacI* gene on an F′ *pro*$^+$ *lac* episome). BU1216 carried the Mu *A* gene mutant Mu *cts*62 *Ats*5045. The λ*c*I 857S7 lysogen was BU851.

**Genetic Procedures.** The media, growth, and induction conditions have been described in detail by Bukhari and Ljungquist (9).

**Biochemical Procedures.**

(*i*) *Extraction of DNA.* The bacterial cells were washed, resuspended in 0.01 M Tris·HCl/1 mM EDTA, pH 7.9, and lysed by the addition of 0.5% sodium dodecyl sulfate. The lysate was digested with Pronase (self-digested for 2 hr at 37° in 0.01 M Tris·HCl, pH 7.4) at a concentration of 1 mg/ml for 8 hr. The solution was then extracted twice with Tris buffer/EDTA/saturated phenol. The aqueous phase was dialyzed against 0.01 M Tris·HCl/1 mM EDTA, pH 7.9, after which it was treated with RNase at a concentration of 100 μg/ml for 2 hr at 37° and then with Pronase at 100 μg/ml for 3 hr at 37°. The DNA was extracted with phenol again and dialyzed as above.

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Abbreviations: *Eco*RI, *Bgl* II, and *Bal* I refer to restriction endonucleases from *E. coli* RY 13, *Bacillus globiggi*, and *Brevibacterium albidum*, respectively.

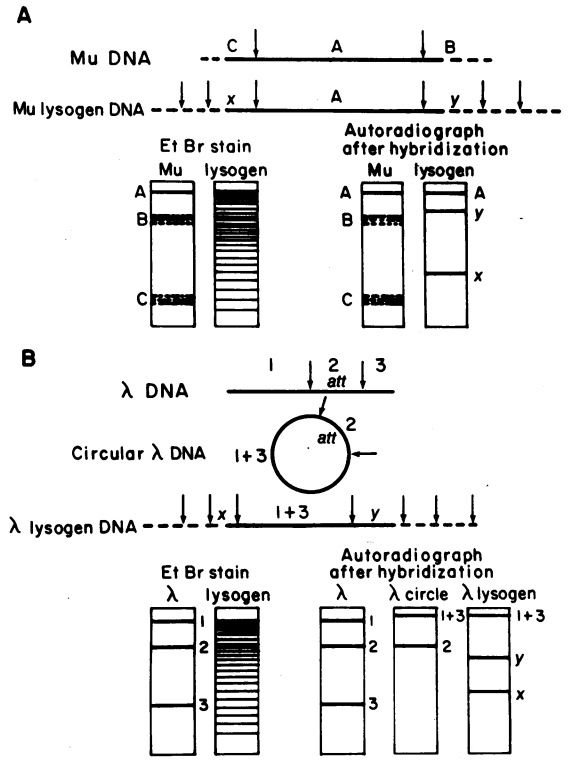


FIG. 1. Scheme for identifying the prophage DNA. Phage DNA is indicated by solid lines and host DNA by broken lines. (*A*) A hypothetical restriction endonuclease cuts mature Mu DNA at two sites (arrows), generating one internal fragment, A, and the two end fragments, B and C, which are replaced in prophage Mu by two prophage–host junction fragments, *x* and *y*. The fragments A, *x*, and *y* will be visible in the autoradiograph after blotting-hybridization of the lysogen DNA fragments with $^{32}$P-labeled Mu DNA as a probe. (*B*) A hypothetical restriction endonuclease cuts linear λ DNA at two sites, generating one internal fragment, 2, and the two end fragments, 1 and 3, which fuse to form one fragment (1 + 3) when λ DNA becomes circular (λ circle). Upon integration, fragment 2 (containing the λ *att* site) is split, giving rise to two host–phage junction fragments labeled *x* and *y* (λ lysogen). Blotting-hybridization with $^{32}$P-labeled λ DNA as a probe will allow the visualization of λ DNA pattern in the λ lysogen DNA fragments.

(*ii*) ***Fractionation, blotting, and hybridization of DNA fragments.*** The DNA was digested with restriction endonucleases as described by Sharp *et al.* (10). The endonucleases used were *Eco*RI (from *E. coli* RY 13), *Bgl* II (from *Bacillus globigii*), and *Bal* I (from *Brevibacterium albidum*). The fragments were resolved by electrophoresis in 1% agarose gels in the presence of ethidium bromide and photographed in ultraviolet light. The fragments were then denatured and transferred directly to a nitrocellulose paper (11) with a procedure referred to here as blotting. The nitrocellulose paper was then coated with Denhardt's solution and hybridized with $^{32}$P-labeled denatured probe DNA by the DNA·DNA hybridization procedure described by Bukhari *et al.*(5). The nitrocellulose paper was then washed, dried, and autoradiographed.

## RESULTS

**Identification of Prophage Mu and Prophage λ DNA.** To study the fate of prophage DNA upon induction, we wished to physically locate the DNA integrated in the host genome. The identification of prophage DNA was based on the following principle. When the total DNA of lysogenic cells is extracted and digested with a restriction endonuclease, the digest will contain fragments of host DNA and phage DNA and two fragments containing the right and left junctions of prophage and host DNA. All the fragments will give rise to a complex pattern or a continuous smear after electrophoresis in agarose gels and staining with ethidium bromide. To identify the fragments containing phage DNA, the fragments in the gels are denatured and blotted onto a nitrocellulose paper and hybridized to $^{32}$P-labeled denatured phage DNA. The bands containing phage DNA will become labeled and can be visualized after autoradiography. A schematic drawing of the expected results for prophage Mu and prophage λ is presented in Fig. 1.

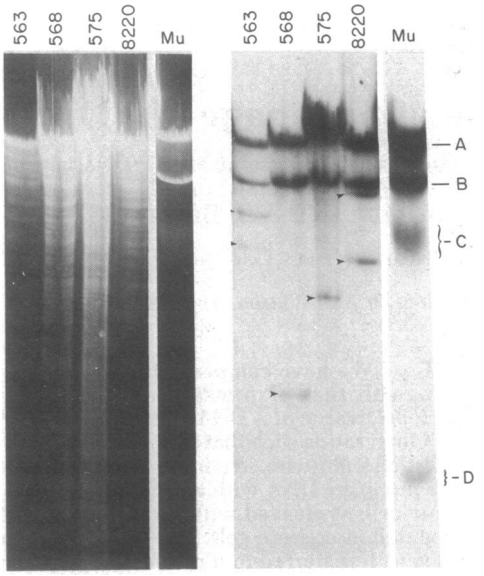


FIG. 2. Hybridization of fragments, generated by *Bal* I digestion of DNA extracted from different Mu lysogens, with $^{32}$P-labeled Mu DNA. (*Left*) Ethidium bromide-stained gel; (*Right*) autoradiograph after blotting-hybridization. The lysogens show one or two new Mu-containing fragments, indicated by arrows.

For Mu, the prophage map and the phage map are collinear. Consequently, the internal phage DNA fragments obtained after digestion with any hypothetical restriction enzyme will be the same for the prophage and the mature phage DNA (fragment A in Fig. 1*A*). Since the ends of Mu are heterogeneous in size and sequence (5), the end fragments from mature phage DNA will appear diffuse or "fuzzy" if the cuts are close to the ends (fragments B and C in Fig. 1*A*). The junction fragments between prophage DNA and host DNA (labeled *x* and *y*) will be sharp bands with mobilities different from those obtained from the mature phage DNA. The junction fragments are expected to be different for prophages located at different sites on the host genome. The results of such an experiment are shown in Fig. 2. The total DNA from different lysogens was digested with *Bal* I. *Bal* I cuts mature Mu DNA at three sites (12), generating two larger internal fragments, A and B, and two smaller end fragments, C and D (see Fig. 4 for the *Bal* I cleavage map of Mu). The end fragments appeared fuzzy in this case because the cuts were close to the heterogeneous ends. The two internal fragments, A and B, were detected in all the lysogens but, as expected, new junction fragments were seen in each lysogen. In some cases, only one new fragment appeared; the other fragment was probably too large to be resolved from the largest internal fragment.

The schematic drawing in Fig. 1*B* shows the fragments expected from prophage λ as compared to circular and mature λ DNA. In λ, one of the internal fragments of the mature phage DNA contains the *att* site (fragment 2). Upon circularization, the end fragments will fuse and give rise to one new fragment

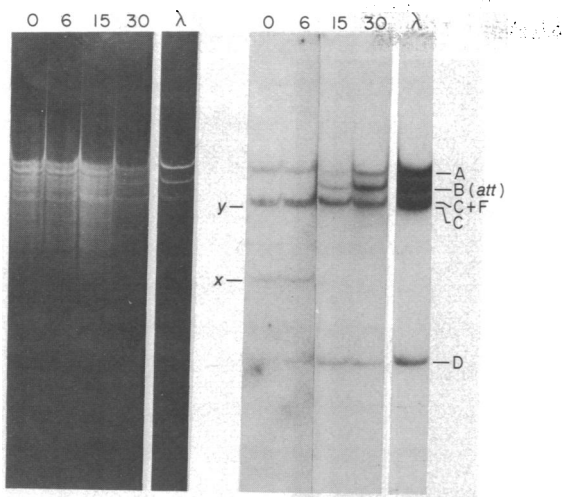


FIG. 3. Hybridization of fragments generated by *Bgl* II cleavage of the λ lysogen (BU851) DNA with $^{32}$P-labeled λ DNA. The DNA was extracted before and after temperature-induction at the times indicated above each slot. Time 0 indicates time of shift to 44°. After 15 min at 44°, the culture was shifted to 37°. (*Left*) Ethidium bromide-stained gel; (*Right*) autoradiograph after blotting-hybridization. The *Bgl* II cleavage map of linear λ DNA is shown at the bottom. Fragment B contains the *att* site; the host–phage junction fragments are represented by *x* and *y*. Fragments E and F are not included.

(fragment 1 + 3). Upon integration, the fragment containing the *att* site will split and give rise to two new junction fragments (fragments *x* and *y*).

**Excision of λ Prophage DNA.** According to the well-established Campbell model, λ DNA is excised from the host chromosome upon induction of a normal λ prophage. To monitor this excision event, we extracted total DNA from a λ lysogen before and during temperature induction. The DNA was digested with restriction endonucleases, and after fractionation by gel electrophoresis the fragments were blotted and hybridized to $^{32}$P-labeled λ DNA. As outlined (Fig. 1*B*), excision

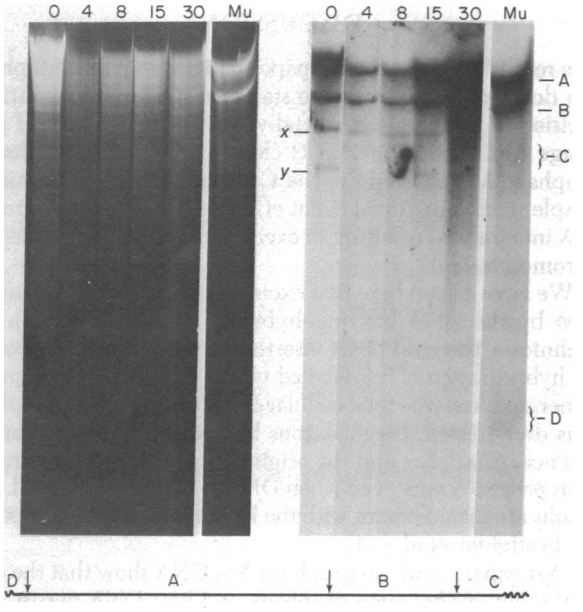


FIG. 4. Hybridization of fragments generated by *Bal* I cleavage of the Mu lysogen (BU563) DNA with $^{32}$P-labeled Mu DNA. The DNA was extracted after temperature induction at the times indicated above each slot. Time 0 indicates time of shift to 44°. After 15 min, cultures were shifted to 37°. (*Left*) Ethidium bromide-stained gel; (*Right*) autoradiograph after blotting-hybridization.

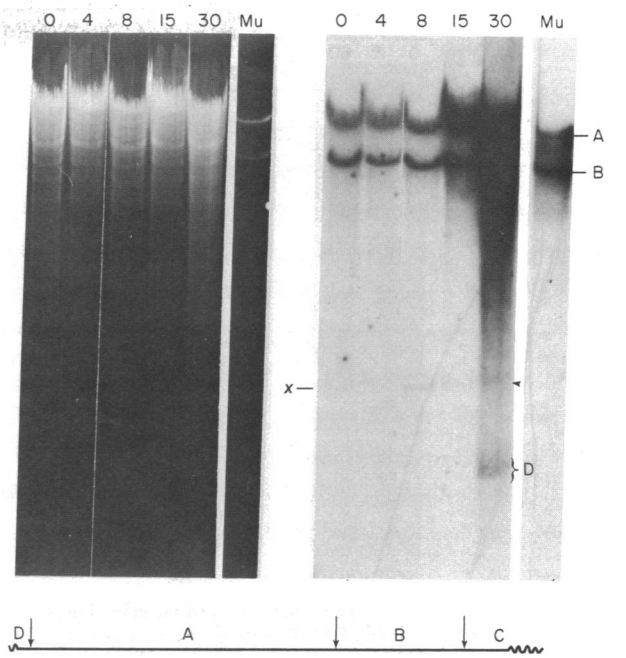


FIG. 5. Hybridization of fragments generated by *Bal* I cleavage of the Mu lysogen (BU568) DNA with $^{32}$P-labeled Mu DNA. The procedure was the same as described for Fig. 4. Only one host–phage junction fragment, *x*, is detected (arrow). (*Left*) Ethidium bromide-stained gel; (*Right*) autoradiograph.

of the prophage as a circular λ DNA molecule can be seen as the disappearance of the junction fragments and the appearance of the fragment containing the *att* site. Fig. 3 shows the results obtained after cleavage with the restriction enzyme *Bgl* II, which cuts mature linear λ into six fragments as indicated. Fragments C and F contain the cohesive or "sticky" ends, and fragment B contains the attachment site (13). Before induction, at time 0, the *att*-containing fragment B was absent and at least one new junction fragment, *x*, could be clearly seen. The other junction fragment, *y*, was probably the one just below the fusion fragment C + F. With time, the excision of λ DNA was observed as a disappearance of fragments *x* and *y* and the appearance of fragment B. Similar results were obtained with the restriction endonuclease *Eco*RI (data not shown). In both cases, disappearance of the junction fragments as well as appearance of the fragment containing the *att* site was seen 15 min after induction. This result is in agreement with the conclusions reached by Freifelder *et al.* with a different technique (14).

**Fate of Prophage Mu DNA upon Induction.** When a Mu *cts*62 lysogenic culture is temperature induced—i.e., the culture is transferred to 44° for 15 min and thereafter incubated at 37°—the phage begins to multiply and the cells are lysed. The total DNA was extracted from lysogenic cells before and at different times after induction. After digestion with the restriction endonuclease *Bal* I, the fragments were blotted and hybridized to denatured $^{32}$P-labeled Mu DNA. Fig. 4 shows the ethidium bromide-stained gels and the corresponding autoradiographs of the fragments obtained after digestion of the DNA of induced cells. Fig. 5 shows the results obtained with another lysogen, BU568. As shown at the bottom of the figures, *Bal* I cut Mu DNA at three sites, giving rise to two larger internal fragments, A and B, and two smaller end fragments, C and D. The autoradiograph of strain BU563 before induction, time 0, showed two new fragments, labeled *x* and *y* (Fig. 4), but only one new fragment, *x*, was detectable in strain BU568 (Fig. 7). These fragments must be the junction fragments between prophage DNA and host DNA. It can be seen from Figs. 6 and

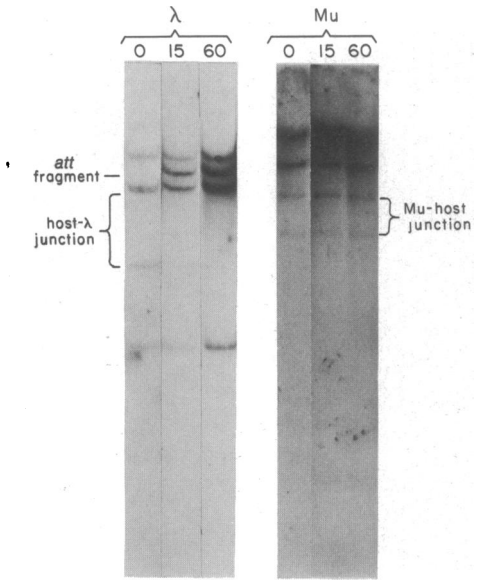


FIG. 6. Induction in the presence of nalidixic acid. The λ lysogen BU851 and the Mu lysogen BU563 were grown to a density of about $1 \times 10^8$ cells per ml. Nalidixic acid was added to a final concentration of 100 μg/ml, and the cultures were shifted to 44°; samples were taken at the times indicated and were treated as in Fig. 3 for λ and as in Fig. 4 for Mu. (*Left*) Autoradiograph of λ DNA samples after blotting-hybridization; (*Right*) autoradiograph of the Mu DNA samples after blotting-hybridization.

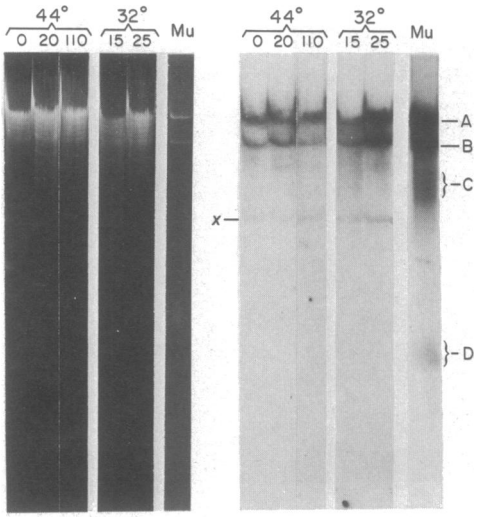


FIG. 7. Hybridization of fragments generated by *Bal* I cleavage of the Mu *cts Ats* lysogen (BU1216) DNA with $^{32}$P-labeled Mu DNA. The procedure was the same as in Fig. 4, except that after 110 min at 44° one part of the culture was shifted to 32°. (*Left*) Ethidium bromide-stained gel; (*Right*) autoradiograph.

7 that the junction fragments remained intact after induction. Their intensity seemed to remain constant. When replication started, the internal fragments increased in intensity and a smear of hybridization appeared as a background. The junction fragments were seen distinctly at 15 min, when Mu DNA had certainly undergone a few rounds of replication. At later times, the fragments began to be covered by the hybridization background. However, the fragments were detectable at 30 min after induction. The background hybridization resulted from continuous integration of Mu DNA at different sites in the lytic cycle, giving rise to random new junction fragments. At the end of the lytic cycle, the mature fuzzy-end fragment D appeared as a consequence of the packaging of phage DNA (Fig. 5). The fact that the original junction fragments persist when replication is well under way can be interpreted to mean that the prophage genome is not excised upon induction. This interpretation would be complicated if the induction process of Mu were very asynchronous. In that case, a similar result might be obtained and the disappearance of the parental junction fragments might go undetected. The induction of prophage Mu, however, appeared to be highly the synchronous, as indicated by the rapid and complete lysis of the culture beginning at 45–50 min after the shift to 44° (data not shown). To further ensure against the possibility of asynchronous induction, we studied the fate of the junction fragments for long periods of time under inducing conditions when DNA synthesis is blocked.

The results obtained when a λ lysogen was temperature-induced in the presence of nalidixic acid (100 μg/ml, which inhibits DNA synthesis) are shown in Fig. 6 *left*. It can be seen that the fragment containing the λ *att* site (see above and Fig. 3) appeared 15 min after induction in the presence of nalidixic acid, and the host–λ junction fragments disappeared. In experiments with prophage Mu (Fig. 6 *right*), no change in the junction fragments was seen. The Mu–host junction fragments present in the uninduced lysogen persisted even after 1 hr at the inducing temperature. No indication of the appearance of any new fragments or mature end fragments was seen under these conditions. Similar results were obtained when the Mu DNA replication was blocked by a phage mutation in gene A, which is required for Mu replication and integration (15, 16). The results obtained after induction of cells lysogenic for Mu *cts Ats* are shown in Fig. 7. This strain gave rise to one detectable junction fragment (labeled *x* in Fig. 7), which was distinguishable from the mature end fragments when digested with *Bal* I. This junction fragment persisted even 110 min after a temperature shift. When the culture was shifted to a permissive temperature, the junction fragment remained but an increase in the intensity of the internal fragments (A and B in Fig. 7) occurred about 25 min after the shift down.

## DISCUSSION

The results presented in this paper indicate that bacteriophage Mu does not conform to the standard mode of integration–excision of temperate bacterial viruses. The behavior of prophage Mu upon induction is clearly different from that of prophage λ. According to the Campbell model, a seemingly simple recombinational event effectively reverses the process of λ integration, resulting in excision of λ DNA from the host chromosome (1).

We have shown here that excision of λ can be monitored *in vivo* by the DNA blotting–hybridization technique. In this technique, the viral DNA inserted in the host DNA is located by hybridization of $^{32}$P-labeled viral DNA with the fragments generated from the total cellular DNA (5, 17). When prophage λ is derepressed, the junctions between phage and host sequences disappear and the original phage *att* site is restored. This process occurs even when DNA synthesis is inhibited. Our results are in agreement with the kinetics of λ excision reported by Freifelder *et al.* (14).

Our experiments on prophage Mu DNA show that the original junctions between prophage and host DNA persist after induction until late in the lytic cycle. The prophage–host junctions remain detectable at least up to 30 min after induction. These results imply that prophage DNA remains at its original location when the lytic cycle is well advanced and Mu DNA is actively replicating. When DNA synthesis is inhibited,

the junctions of Mu prophage and host remain intact for long periods of incubation under conditions of prophage derepression. In the case of a Mu *cts Ats* mutant, no Mu replication is detected at the nonpermissive temperature. However, 25 min after a shift to permissive temperature, replication of Mu DNA is detected. These results confirm that the *A* gene product is required for normal Mu DNA replication.

Many copies of Mu DNA are known to be integrated at different sites in the host genome during the lytic cycle (2, 18). We find that by 30 min after prophage induction so many new junction fragments have been generated that a smear of hybridization activity is obtained on the autoradiographs. No Mu ends free of host sequences are detected. Toward the end of the lytic cycle the mature ends of Mu DNA begin to appear, indicating the beginning of the morphogenetic processes. If the original Mu–host junction fragments are cleaved after 30 min of induction, they would not be detectable because of the extensive hybridization background. A change in the junction fragments might occur if the prophage DNA is cut for packaging. Packaging of Mu DNA apparently occurs from maturation precursors that contain Mu DNA covalently linked to host DNA (19).

That Mu DNA does not appear to be excised upon prophage induction, and yet many copies of Mu DNA are inserted into the host genome, implies that prophage DNA remains at the original site. Therefore, we postulate that the Mu integrative precursor, the form of DNA that is inserted into the host DNA, is generated by replication of Mu DNA. This hypothesis is consistent with our observation that the parental Mu DNA after infection of host cells is not efficiently integrated into host DNA and thus must be replicated before integration (E. Ljungquist and A. I. Bukhari, unpublished data).

If Mu DNA replicates *in situ*, without having been excised, then the replication might be expected to proceed into the host sequences adjacent to the prophage DNA. In recent experiments by B. Waggoner and M. Pato (personal communication) and by us (unpublished data), no extensive amplification of the host sequences adjacent to the prophage could be detected after induction. These observations imply that a mechanism exists for the recognition of the specific Mu ends, such that replication does not penetrate the Mu–host junction. It should be noted that the heterogeneous host sequences at Mu ends are not inserted into the host chromosome during integration of Mu DNA after infection. Absence of replication of adjacent host sequences after prophage Mu induction suggests a mechanism by which the host sequences at the phage ends are left out during integration. If infecting phage DNA first replicates, leaving out the host sequences, the resulting integrative precursor will be a form devoid of host sequences. Such a form, however, has not been observed. It may be that the integrative precursor of Mu has a very short half-life and is quickly integrated or that the replication of Mu DNA is coupled to its integration.

We would like to point out that Mu integration after prophage induction is reminiscent of translocation of the transposable elements. The transposable elements can be excised at an apparently low frequency ($10^{-5}$–$10^{-7}$) from a specific site in a gene, and yet their transposition frequencies are higher by a factor of $10^3$–$10^4$ (ref. 7). When reversion of mutations caused by the insertion of translocatable elements (or transposons) is examined, almost all of the revertants are found to have lost the transposon altogether (20, 21). Thus, excision of these elements does not generally lead to their integration. A similar phenomenon is seen in bacteriophage Mu. Prophage Mu under certain conditions (Mu *cts* prophages carrying the *X* mutation) can be excised at a frequency of $10^{-5}$–$10^{-7}$ and is lost from the cells (22, 23). As the experiments reported in this paper clearly indicate, this type of Mu excision does not occur efficiently upon induction. Thus, although a mechanism for prophage Mu excision exists, this mechanism does not appear to be involved during the normal Mu life cycle. In view of the mechanistic similarity, we propose that Mu is a representative of transposable elements. These elements constitute a class of insertion elements, different from most temperate phages, in which replication of the inserting molecule is a necessary step in the process of its integration.

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