DNA REARRANGEMENTS ASSOCIATED WITH REVERSION OF BACTERIOPHAGE MU-INDUCED MUTATIONS

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ABSTRACT

Excision of transposable genetic elements from host DNA is different from the classical prophage lambda type of excision in that it occurs at low frequency and is mostly imprecise; only a minority of excision events restores the wild-type host sequences. In bacteriophage Mu, a highly efficient transposon, imprecise excision is 10–100 times more frequent than precise excision. We have examined a large number of these excision events by starting with MuX mutants located in the Z gene of the lac operon of Escherichia coli. MuX mutants are defective prophages whose excision occurs at a measurable frequency. Imprecise excision was monitored by selecting for melibiose+ (Mel+) phenotype, which requires only a functioning lacY gene. Mel+ revertants exhibit an array of DNA rearrangements and fall in four main classes, the predominant one being comprised of revertants that have no detectable Mu DNA. Most of these revertants can further revert to Lac+. Perhaps 5 base-pair duplications, originally present at prophage-host junctions, are left in these lacZ-Y+ revertants, and they can be further repaired to lacZ+. Another class has, in addition to the loss of Mu DNA, deletions that extend generally, but not always, to only one side of the prophage. The other two classes of revertants, surprisingly, still have Mu DNA in the lacZ gene. One class has deletions in the Z gene, whereas, no deletions can be detected in the other. Many of the revertants in the last class can further revert to lacZ+, indicating that the lacY gene must have been turned on by a rearrangement within Mu DNA. Apparently, all of the detectable precise and most of the imprecise excision events require functioning of the Mu A gene. We suggest that a block in large-scale Mu replication allows the excision process to proceed.

THE temperate bacteriophage Mu is a transposon; its genome consists of about 37,000 nucleotide pairs and contains genes for replication-transposition, as well as for phage morphogenesis (see BUKHARI 1976). The repressor gene c located at the left end controls expression of the A and B genes of Mu needed for replication and transposition. Upon induction, a Mu prophage replicates and undergoes transposition to different sites on the host DNA. Upon infection of sensitive Escherichia coli cells, the phage DNA is integrated at apparently randomly distributed sites on the host genome. Lysogens isolated after infection contain mutations at a high frequency, caused by the insertion of Mu DNA (TAYLOR 1963). Mutations induced by Mu are nonleaky, polar and extremely stable. The

frequency of reversal of wild-type Mu integration is less than $10^{-10}$ (Taylor 1963).

It was reported earlier that Mu integration, in fact, can be reversed by first isolating prophage mutants unable to kill the host cells upon induction (Bukhari 1975; Bukhari and Froshauer 1977). These so-called $X$ mutants are found among the heat-resistant survivors when cells lysogenic for temperature-inducible Mu (Mucts) are plated at high temperature. The $X$ mutants, by definition, are defective prophages that can be excised at measurable frequencies. The mutations in $X$ mutants examined so far have been found to be mainly insertions in the $B$ gene (Khatoon and Bukhari unpublished). Thus, whereas a Mucts prophage is $A^+ B^+$, $X$ mutants are $A^+ B^-$ (and presumably do not express several other early genes of Mu because of polar effects; see Giphart-Gassler and van de Putte 1979). $X$ mutants are no longer able to replicate their DNA or to express their morphogenetic functions. In effect, they have lost the functions that make Mu a virus.

Excision of Mucts $X$ mutants has been monitored from the lacZ gene of E. coli. The excision is a low-frequency event and is mostly, although not always, imprecise, giving rise to $Z^-$ but phenotypically $Y^+$ revertants. Many other transposable elements have been shown to be excised both precisely and imprecisely, with precise excisions normally being the much rarer of the two events. Transposable elements such as Tn10 (Botstein and Kleckner 1977) and Tn5 (Berg 1977) exhibit such excision behavior. Understanding the mechanisms by which transposable elements can be excised from host DNA may be important in understanding the general principles by which DNA rearrangements occur. Excision mechanisms are particularly intriguing since transposition of Mu and other transposable elements may involve replication rather than excision of the element (Ljungquist and Bukhari 1977).

To study the process of Mucts $X$ excision from the lacZ gene, we have analyzed large numbers of Mel$^+$ (imprecise excision events) and Lac$^+$ (precise excision events) revertants by fine genetic mapping and by DNA-DNA hybridization. The results of these studies are summarized in this paper. An astonishing array of DNA rearrangements can be seen when Mucts $X$ mutants are plated for Mel$^+$ revertants. Most Mel$^+$ revertants have undergone Mu excision, deleting Mu DNA and, in general, deleting host DNA adjacent to one side of the prophage. Other Mel$^+$ revertants, however, still retain Mu sequences, and yet expression of the lacY gene is no longer restricted. Most precise and imprecise excision events require Mu $A$ gene function.

**MATERIALS AND METHODS**

**Bacterial and phage strains:** All the strains used were derivatives of E. coli K12 and are listed in Table 1. Strains carrying standard deletions and point mutations were from the collection of D. Zipse, and most of them are described elsewhere (Zipse et al. 1970; Bukhari and Zipse 1972; Bukhari 1975). Positions of the standard deletions and point mutations of the strains not described earlier can be seen in Figures 1, 6, 8, 10 and 12.

The bacteriophage strains used were Muc$^+$ (Taylor 1963), Mucts62, a temperature-inducible mutant owing to a mutation in the repressor gene $c$ (Howe 1973), Muc3, a clear-plaque
mutant (Bukhari and Metlay 1973), D108 cts (Hull, Gill and Curtis 1978) and φ80 SuIII (Fianit et al. 1971).

**Media and growth conditions:** Nutrient media (LB broth or agar), minimal media (M9 salts supplemented with a sugar and other nutrients), methods for cultivation of phage and procedures for construction of lysogens have been described by Bukhari and Ljungquist (1977). To select for Lac+ revertants, cells were plated on minimal medium with lactose as the sole carbon source at 37°C, unless specified otherwise. Lac+ revertants were also isolated from McConkey-lactose differential medium, in which Lac+ colonies have a growth advantage over Lac− colonies, particularly when cell density is high. To select for Mel+ revertants, cells were plated on minimal medium with melibiose as the sole carbon source at 41-42°C. At this temperature, melibiose is transported into the cells solely by lac permease, the product of the lacY gene (see Beckwith 1970).

**Genetic nomenclature:** Lac+ (lactose utilization) and Mel+ (melibiose utilization) are phenotypic symbols; the genotypes are represented by gene symbols lacZ or lacY. Su+ is a phenotypic symbol indicating that the strain is able to suppress the nonsense mutation.

**Isolation of Mu X mutants, Mu cts amber insertions and Mu cts amber mutants:** The X mutants of Mu cts prophage, located in lacZ gene on an F'pro+lac episome, were obtained by plating approximately 5 x 10⁸ cells of a lysogen (Mu cts62, MuR) at 42°C and screening the survivors for reversion to Lac+ as described by Bukhari (1975). Reversion to Mel+ and Lac+ was studied in the original Mu-resistant host at 43°C and 37°C, respectively. In cases where the X mutants were located on the episome, Lac+ and Mel+ revertants were tested for Mu sensitivity or resistance after transfer of the episome to a Mu-sensitive host, BU7026. Isolation of insertions of Mu cts62 carrying an amber mutation in gene A or gene B was carried out as follows. The F'pro+lac+carrying strains (FPL5014 or BU1687), containing an amber suppressor (which, in the case of BU1687, was provided by lysogenizing the cells with φ80 SuIII), were infected with Mu cts62 Aam1093 or Mu cts 62 Bam1066. Lac− lysogens (with Mu am insertion in lac) were picked, checked for phage production on an Su+ indicator (BU1532) and conjugated with a Mu-resistant Su+ strain (BU1683 or BU1684) in order to transfer the episome into a Mu-resistant background. Simultaneous transfer of the episome and the Mu prophage confirmed the presence of a Mu insertion in the episome. The strains so constructed (BU1703 and BU1705) were used to isolate the X mutants by the usual procedure. Two independent X mutants, A1093-X1 and A1093-X2, were isolated from the Mu cts62 Aam1093 insertion, and three X mutants, B1066-X1, B1066-X2 and B1066-X3 (strains Bu1715a, b and c, respectively), were isolated from the Mu cts62 Bam1066 insertion. The location of Mu cts A1093 (and its X mutants) in the lacZ gene was mapped between lac deletions 18 and 237, while that of Mu cts B1066 (and its X mutants) was mapped between Δ22 and Δ253 (for positions of these deletions, see Figure 1).

To examine excision of Mu cts X amber mutants in Su− background, the F' episomes carrying the X mutants were transferred to a Mu-resistant, Su− host, BU1547.

**Genetic mapping:** Fine genetic mapping of the lacZ gene was carried out by matings on McConkey's lactose medium, using emergence of Lac+ colonies as an index of recombination. Donor and recipient cells were mixed on plates and incubated for 48 hr or more at 37°C or 32°C. Controls of unmated donor and recipient cells were always made. Occasionally, the matings were also done by replica plating; the clones containing mutations to be mapped were replica plated onto a lawn of a strain carrying a standard deletion or mutation.

**Biochemical procedures:** Total cell DNA from the lysogenic bacteria was prepared according to the procedure described by Ljungquist and Bukhari (1977). The DNA was digested with the restriction endonuclease BalI (from Brevibacterium albidum) in a mixture containing 6 mM Tris-HCl (pH 7.9), 6 mM MgCl₂ and 6 mM β-mercaptoethanol, as described by Sharp, Sugden and Sambrook (1973). Restriction fragments were resolved by electrophoresis at 50-60 mA on 1% agarose gel in the presence of ethidium bromide (1 µg/ml) and photographed under UV.

For hybridization, the restriction fragments separated by electrophoresis were denatured and transferred to nitrocellulose paper by the Southern (1975) procedure. The transfer of denatured fragments to a nitrocellulose paper will be referred to here as "blotting." The nitrocellulose paper was then dried, baked under vacuum at 80°C for 6 hr and soaked overnight in Denhardt's solution...
# Bacterial Strains

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<th>Source of derivation</th>
<th>Genotype and relevant markers</th>
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</tr>
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<td>40 lysogenized with D108cts</td>
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</tr>
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<td>BU1525 × BU1621</td>
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<td>BU1508a × BU1621</td>
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<td>This work</td>
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<td>BU1705</td>
<td>F'pro⁺lacZ::(Mučts62 Aam1093)/Δ prolac, his-11, met-5, StrR, MuR, φ80 SuIII</td>
<td>BU1694 × BU1683</td>
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<td>BU1724</td>
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<td>BU1715a</td>
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<td>X mutant from BU1703</td>
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<td>BU1715b</td>
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<td>X mutant from BU1703</td>
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<td>BU1715c</td>
<td>F'pro⁺lacZ::(Mučts62 1066-X3)/Δ prolac, trp-8, MuR, φ80 SuIII</td>
<td>X mutant from BU1703</td>
</tr>
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</table>

MuR = resistant to bacteriophage Mu; NaI² = Nalidixic acid resistant; StrR = streptomycin resistant. Trp-8 is an amber mutation in the trpA gene. φ80 SuIII carries gene supF', which suppresses an amber mutation by inserting tyrosine.
Hybridization was carried out according to the method described by BUKHARI, FROSHAUER and BOTCHAN (1976), using a DNA probe labeled with $^{32}$P, by the "nick-translation" method of MANIATIS, JEFFREY and KLEID (1975).

RESULTS

Characterization of Mu prophages in the lacZ gene and their X derivatives: We have previously described several Mucts62 insertions in the lacZ gene that were used to study excision of Mu DNA (BUKHARI 1975). These insertions are polar; in addition to the lacZ gene, the lacY and A genes are not expressed. In the present study, we used insertions 8305, 8306, 8354, 8357 and 8358. All of these insertions of Mucts62 are carried in an F$^+$pro+lac episome and therefore can be readily transferred to different genetic backgrounds. The positions of these insertions were mapped against a set of 27 deletions. The refined map of these insertions, indicating their locations and orientations, is given in Figure 1. We isolated the X mutants from the strains BU8305, BU8306, BU8354, BU8357 and BU8358 by plating these strains at 43°C. The X mutants, by definition, are the defective prophages that can be excised. One X mutant from each strain (8305-X, 8354-X, 8357-X and 8358-X) was chosen for a detailed analysis of Mu DNA excision. The Mucts62 X prophages were remapped genetically; in each case the location of the X mutant coincided with the location of the parent prophage.

In addition to the mapping, we characterized the Mu prophages and their X derivatives physically by restriction endonuclease cleavage of cellular DNA and DNA-DNA hybridization (Figures 2 and 3). Total cell DNA extracted from the lysogenic strains was digested with the endonuclease B*I, blotted and hybridized against $^{32}$P-labeled Mu DNA probe. The Mucts prophages showed two major bands, a and b (Figure 2), representing the two internal Mu fragments obtained by the B*I cleavage of mature Mu DNA (Figure 3, slot 6). These bands originate by the cleavage of sites i and ii, and ii and iii, respectively (see line drawing in Figure 3). Mature Mu DNA, when cleaved with B*I, also yields two end fragments (c and d) that originate by the cleavage of sites i and iii, respectively.

![Figure 1](https://academic.oup.com/genetics/article/98/1/1/5995103)
MU-INDUCED MUTATIONS

FIGURE 2.—Hybridization of the fragments, generated by BalI digestion of the DNAs extracted from Mucts lysogens (BU8305, BU8357, BU8354, BU8305 and BU8358) with $^{32}$P-labeled Mu DNA. Left, ethidium bromide stained gel (1% agarose); right, autoradiograph after blotting hybridization. Fragments a and b represent the internal Mu fragments, and the fragment f, a junction fragment.

(Figure 3), and appear as diffuse bands on the gel (slot 6, Figure 3). The diffuse appearance results from host sequences of different sizes being attached to the ends of mature Mu DNA (Bukhari, Froshauer and Botchan 1976). In the prophage state, where Mu is integrated in the host chromosome, the internal fragments (a and b) are the only ones that are clearly detectable in all the prophages, irrespective of the prophage locations in the host chromosome (Figure 2). The end fragments (c and d), because they are linked to different host sequences, do not band at their respective places (Ljungquist and Bukhari 1977). Their size depends upon the presence and location of BalI sites in the adjacent host sequences. These junction fragments seem to be generally too large to be resolved from the largest internal fragments (a) under our conditions. In some cases, however, a junction fragment (fragment f, Figure 2 and 3) representing one end of the prophage plus the adjacent host sequence could be seen.

In the case of Mucts X prophages, one extra band, e, in addition to the bands a and b, was observed (Figure 3). Band e represents an internal fragment origi-
FIGURE 3.—Hybridization of the fragments, generated by BalI digestion of the DNAs extracted from Mucts X lysogens (8305-X1, 8357-X1, 8354-x1, 8306-X1 and 8358-X1) with 32P-labeled Mu DNA. Left, ethidium bromide stained gel; right, autoradiograph after blotting hybridization. The line drawing represents BalI cleavage sites (i, ii, iii and iv) on Mucts and Mucts X DNAs. a, b, c, d, e and f represent cleavage fragments. Fragment e, which bands at different sites in different X mutants, is marked with arrows. In all subsequent figures, the fragments are identified with letters as shown in this figure.

nating by cleavage at site iv (Figure 3), imparted by the insertion of IS1 in the early genes of Mu (De Brujin and Bukhari 1978; Khatoon and Bukhari 1978). Since the site of insertion could vary within a small region in different X mutants, the size of fragment e also varies from one X mutant to another (Khatoon and Bukhari 1978). All the X mutants used here have been shown to have IS1 insertions by DNA-DNA hybridization. The X mutations can also be caused by IS5 and IS2 insertions (Khatoon and Bukhari, unpublished).

Frequencies of precise and imprecise excision of the Mucts X mutants: As described previously, reversion to Lac+ of a strain carrying the Mucts62 X
MU-INDUCED MUTATIONS

A mutant prophage in the lacZ gene was used as an index of precise excision of Mu DNA; reversion to Mel+ was used as an index of imprecise excision (Bukhari 1975). Fermentation of lactose (Lac+ phenotype) requires the functioning of both the lacZ and the lacY gene; whereas, fermentation of melibiose (Mel+ phenotype) at 41° requires only the functioning of lacY gene. Thus, Lac+ revertants can be assumed to be genotypically lacZ+Y+ (with perhaps some rare exceptions); whereas Mel+ revertants could either be lacZ+Y+ or lacZ-Y+.

We determined the frequencies of precise and imprecise excision of 8305-X1, 8357-X1, 8354-X1, 8306-X1 and 8358-X1. In all cases, frequencies of precise excision were 10-fold (and in some experiments as much as 100-fold) lower than frequencies of imprecise excision. The frequency of precise excision ranged from $1 \times 10^{-6}$ to $1 \times 10^{-8}$ and that of imprecise excision ranged from $1 \times 10^{-5}$ to $1 \times 10^{-7}$. From among the Mel+ (Lac-) revertants, more than 50% could revert further to yield Lac+ colonies.

Excision as a Mu-specific function: Excision of the Mucont X mutant 8305-X1 and 8354-X1 was examined in host strains carrying a Muc+ (wild-type Mu) prophage or D108cts as a prophage. D108 is a heteroimmune Mu phage (Hull, Gill and Curtiss 1978). Table 3 shows that Lac+ reversion (i.e., precise excision) was reduced below the detection level when the host strain carried a secondary Mu prophage. D108cts did not inhibit the precise excision, although there was some reduction in frequency. The noteworthy point is that the presence of a secondary Muc+ prophage did not completely inhibit reversion to Mel+, but caused a 10- to 100-fold reduction in its frequency. Thus, some of the events that lead to Mel+ reversion are not under the control of Mu immunity.

Preliminary studies had shown that the gene A function of Mu was required for excision (Bukhari 1975). To understand the roles of the A and B genes involved in Mu integration and replication, we isolated insertions of Mucont62 Aam1093 and Mucont62 Barn1066 in the lacZ gene carried on an F'pro+lac episome in the Su+ background. Two independent X mutants from the A1093 in-

<table>
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<th>(1)* recA+</th>
<th>(2)* recA-</th>
<th>(3)* recA+</th>
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<td>Mel+</td>
<td>Lac+</td>
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<tr>
<td>8305-X1</td>
<td>$2 \times 10^{-6}$</td>
<td>$6 \times 10^{-7}$</td>
<td>$1.3 \times 10^{-6}$</td>
</tr>
<tr>
<td>8354-X1</td>
<td>$1 \times 10^{-5}$</td>
<td>$1 \times 10^{-7}$</td>
<td>$2 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

* (1) in the recA+ host BU8538; (2) in the recA- host BU8559, and (3) after being transferred back to BU8538 from BU8559.
TABLE 3
Excision of Mucts X mutants in the presence of a secondary Mu or D108 prophage

<table>
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<tr>
<th>Strain</th>
<th>Relevant characteristics</th>
<th>No. of cells plated</th>
<th>No. of Lac+ colonies</th>
<th>No. of Lac-, Mel+ colonies</th>
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<td>F'prolacZ::8305-X1</td>
<td>5 × 10⁸</td>
<td>300</td>
<td>~1000</td>
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<td>~6000</td>
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<td>3 × 10⁸</td>
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<td>n.d.†</td>
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* The complementation group for am6002 has not been determined.
† n.d. = not done.

sition and three from the B1066 insertion were isolated and their excision behavior was examined in both Su+ and Su- backgrounds. The A1093-X1 and A1093-X2 mutants showed precise excision (reversion to Lac+) only in the Su+ background (Table 4). The X mutants carrying the amber mutation 1066 in the B gene could be excised precisely in both Su+ and Su- backgrounds. A1093-X1 and A1093-X2 yielded approximately 1000 Mel+ revertants per 5 × 10⁸ cells each in the Su+ background, but yielded only 98 and 148 Mel+ revertants, respectively, in the Su- background. Such an effect was not found in the case of the X mutants carrying a mutation in the B gene. It would seem, therefore, that the A gene is required for Mu excision, but that certain events leading to Mel+ reversion are independent of the A gene. Thus, A mutations and a secondary Muc+ prophage affect excision events in a similar manner.

Characterization of Lac+ revertants: All Lac+ revertants lose Mu immunity, indicating that Mu DNA is not inserted elsewhere after excision. To confirm this conclusion, DNA of Lac+ revertants was examined for Mu sequences by blotting and hybridization with ³²P-labeled Mu DNA. An example of such an experiment is shown in Figure 4. No Mu sequences were detected in several Lac+ revertants examined.

Characterization of Mel+ Lac- revertants: Mel+ Lac- revertants have the genotype lacZ-Y+. It is critical to understand the lesions left in the Z gene as a

TABLE 4
Reversion to Lac+ of Mucts X mutants carrying amber mutations

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>X mutant</th>
<th>Location of amber mutation on the X prophage</th>
<th>Lac+ reversion in Su+ background*</th>
<th>Lac+ reversion in Su- background?</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU1723</td>
<td>A1093-X1</td>
<td>gene A</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>BU1724</td>
<td>A1093-X2</td>
<td>gene A</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>BU1715a</td>
<td>B1066-X1</td>
<td>gene B</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BU1715b</td>
<td>B1066-X2</td>
<td>gene B</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BU1715c</td>
<td>B1066-X3</td>
<td>gene B</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 4.—Hybridization of the fragments, generated by BalI digestion of the DNAs extracted from lac+ revertants of 8306-X1 and 8354-X1 with 32P-labeled Mu DNA. Only the autoradiograph after blotting hybridization is shown.

consequence of Mu excision. A total of 214 Mel+ revertants, including 65 from 8305-X1, 40 from 8357-X1, 50 from 8354-X1, 19 from 8306-X1 and 40 from 8358-X1 were isolated and studied. In 75% of these Mel+ revertants (161/214), at least a part of Mu DNA had been excised as tested by transferring the F' pro+ lacZ- episomes to a Mu-sensitive host BUX7026 and testing for Mu immunity. Surprisingly, 25% of the Mel+ revertants still retained Mu immunity. Thus, these lacZ-Y+ revertants contained Mu sequences. Both lacZ-Y+ (Mu-) and lacZ-Y+ (Mu+) revertants were screened for deletions by crossing them with a set of appropriate lacZ deletions and point mutations. The Mel+ revertants were also examined to see whether they could revert further to Lac+.

The results are given in Figure 5. As can be seen in the flow sheet, a large majority of Mel+ revertants in which no deletion could be detected could revert to Lac+. For the sake of discussion, the Mel+ revertants will be divided into four classes:
Class I: the Mel+ revertants that had lost Mu DNA and carried deletions of various sizes on one or both sides of the point of insertion.

Class II: the Mel+ revertants that carried parts of Mu DNA insertion with deletions. (Deletions extended only on one side of the point of insertion).

Class III: the Mel+ revertants that retained Mu DNA or its parts and carried no detectable deletions.

Class IV: The Mel+ revertants that had lost Mu DNA and carried no detectable deletions.

All the Class I and Class II revertants, 12 from Class III and 6 from Class IV were studied in detail. The \(\text{lacz}\) mutation in each revertant was finely mapped by using a large set of deletions and point mutations. Furthermore, the arrangement of Mu sequences, if present, was examined in each revertant by DNA-blotting hybridization techniques. The findings are reported below.

(i) Mel+ Lac- revertants of 8305-X1: Twelve Mel+ (Lac-) revertants carrying deletions in the \(\text{lacz}\) gene and eight without any detectable deletions were studied. Fine genetic mapping of these Mel+ revertants is shown in Figure 6, and studies involving DNA-DNA hybridization are presented in Figure 7. On the basis of these studies, the Mel+ revertants could be placed into the above-mentioned four classes as follows:
Class I included the Mel+ revertants Nos. 1, 4, 6, 8, 9, 15, 16, 17, 19 and 20 (Figure 6). All of them carried deletions of various sizes in their lacZ gene. The deletions of Nos. 9, 17 and 19 extended to both sides of the point of insertion; those of Nos. 4, 6, 8, 15, 16 and 20 extended to the right and that of No. 1 extended to the left side of the point of insertion. None of them transferred Mu immunity when conjugated to BUX7026, and those examined (Nos. 1, 15, 19 and 20) did not show hybridization with 32P-labeled Mu DNA (Figure 7).

Class II included two Mel+ revertants, Nos. 5 and 7. They showed large deletions in the lacZ gene on one side of the point of insertion and showed hybridization with 32P-labeled Mu DNA. Most of the Mu DNA had been deleted; only the junction fragment could be seen clearly.

Class III included seven Mel+ revertants, Nos. 2, 3, 10, 12, 13, 14 and 18. They had no detectable deletions in the lacZ gene, conferred Mu immunity when con-
FIGURE 7.—Hybridization of the fragments generated by *BalI* cleavage of the DNAs extracted from *Mel*⁺ and *lac*⁺ revertants of 8305-X1 with "P-labeled Mu DNA. Autoradiograph after blotting hybridization is shown. Wild-type *E. coli* control was Mu⁻.

jugated to a Mu-sensitive host, BUX7026, and showed hybridization with Mu DNA. Mel⁺ revertants 3, 10, 12 and 18 could be clearly seen to have retained most, if not all, of the X prophage; whereas, in revertant No. 2, the internal Mu fragments appeared to be missing (Figure 7). Mel⁺ revertants Nos. 2, 3, 10, 12 and 18 were also found to revert spontaneously to yield Lac⁺ colonies, the episomes of which no longer conferred Mu immunity in BUX7026. These Lac⁺ revertants showed no hybridization with "P-labeled Mu DNA.

The only class IV Mel⁺ revertant examined was No. 11. It had no detectable deletion in its lacZ gene and did not confer Mu immunity on BUX7026. Also, it was not able to revert further to Lac⁺.

(ii) Mel⁺ Lac⁻ revertants of 8357-X1: Among the Mel⁺ Lac⁻ revertants of 8357-X1, 12 carrying detectable deletions and three not carrying detectable deletions in the lacZ gene were chosen to study. Fine genetic mapping of these Mel⁺ revertants is shown in Figure 8, and DNA-DNA hybridization studies are shown in Figure 9. On the basis of genetic and biochemical studies, the Mel⁺ revertants were placed into the four classes as follows:

Class I included Mel⁺ revertants Nos. 1, 3, 5, 8, 9, 11, 12, 14 and 15. Of these,
Nos. 5, 9, 11, 14 and 15 carried deletions on both sides of the original insertion site; Nos. 1, 8 and 12 carried deletions extending to the right and No. 3 carried deletions extending to the left side of the insertion site. None of the Mel+ revertants, when conjugated to BUX7026 (Mu-sensitive host), transferred Mu immunity with the episome, and those tested (Nos. 1, 5 and 15, Figure 9) did not show Mu DNA upon hybridization. Class II included Mel+ revertants Nos. 2, 7 and 13, all of which carried deletions extending to only one side of the point of insertion. Mel+ revertants Nos. 2 and 7 showed Mu DNA upon hybridization with $^32$P-labeled Mu DNA, and No. 13 transferred Mu immunity with the episome. Class III included one Mel+ revertant, No. 4. It had no detectable deletion in the lacZ gene and transferred Mu immunity with the episome. Class IV Mel+ revertants were Nos. 6 and 10, both of which had no detectable deletion in the lacZ gene. None of them transferred Mu immunity and Mel+ No. 10 did not show Mu DNA upon hybridization with $^32$P-labeled Mu DNA. Both were able to revert further to yield Lac+ colonies.

(iii) Mel+ Lac- revertants of 8354-X1 and 8306-X1: 8354-X1 and 8306-X1 are situated very close to each other in the lacZ gene (Figure 1). Mel+ Lac- revertants of these X mutants are therefore numbered in the same sequence (Figure 10), the first ten Mel+ revertants (1–10) being of 8354-X1 and the last five (11–15) of 8306-X1. The Mel+ revertants examined were as follows:
Class I included Mel+ revertants Nos. 1, 2, 3, 4, 5, 6, 8, 10, 12 and 15. They all carried deletions of various types in the \( lac^+ \) gene (Figure 10) and lacked Mu DNA as indicated by the absence of transfer of Mu immunity with the episome. Mel+ revertants Nos. 1, 2, 8, 10 and 12 also showed absence of Mu DNA when tested by DNA-DNA hybridization (Figure 11). Class II included one Mel+ revertant, No. 9. It had a deletion in the \( lac^+ \) gene and seemed to have retained most of the Mu DNA. It could transfer Mu immunity with the episome. Class III included Mel+ revertants Nos. 11, 13 and 14, all of which carried Mu DNA without any detectable deletions in the \( lac^+ \) gene. Mel+ revertants Nos. 11 and 14 reverted further to yield Lac+ colonies. Loss of Mu DNA from the Lac+ revertants could be shown by DNA-DNA hybridization. Mel+ revertant No. 7 was in class IV because it had no detectable deletion in the \( lac^+ \) gene and lacked Mu DNA. It was also able to revert further to yield Lac+ colonies.

(iv) Mel+ Lac- revertants of 8358-X1: Of the Mel+ Lac- revertants of 8358-X1, 18 carrying detectable deletions and three without any detectable deletions in the \( lac^+ \) gene were studied in detail. Fine genetic mapping of these Mel+ revertants is shown in Figure 12, and DNA-DNA hybridization studies are shown in Figure 13. The Mel+ revertants were placed into the four classes as follows:

![Figure 9](image-url)
Figure 10.—A map of the lesions in the lacZ gene of some selected Mel+ revertants of the X mutants 8354-X1 and 8306-X1 of BU8354 and BU8306, respectively. The number (1-10, 11-15) listed on the right-hand side represent Mel+ revertants of 8354-X1 (1-10) and 8306-X1 (11-15).

Class I included Mel+ revertants Nos. 1, 2, 4, 6, 8, 10, 15, 16, 17, 18, 19 and 20. None of the Mel+ revertants transferred Mu immunity with the episome, and each of them carried a deletion in the lacZ gene. Those examined (Nos. 1, 2, 4, 10 and 18) by DNA-DNA hybridization also showed absence of Mu DNA. Class II included Mel+ revertants Nos. 7, 11, 12, 13 and 14, carrying deletions in the Z gene. All of them carried Mu DNA as shown by DNA-DNA hybridization and transferred Mu immunity with the episome. Some variations in Mu sequences were seen. For example, in revertant 7, a new fragment appears underneath the second internal Mu fragment, and in No. 12, the junction fragment is smaller. Class III included Mel+ revertant No. 3. It had no detectable deletion in the lacZ gene, carried Mu DNA and could revert further to yield Lac+ colonies. Class IV included Mel+ revertants Nos. 5 and 9. They did not transfer Mu immunity with the episome and carried no detectable deletions in the lacZ gene. Mel+ revertant No. 5 showed absence of Mu DNA when examined by DNA-DNA hybridization. Both of them revert further to yield Lac+ colonies.

(v) General remarks on the mapping data: The data presented above on fine
genetic mapping and DNA-DNA hybridization do not reflect a random sampling of Mel+ revertants. For fine genetic mapping, Mel+ revertants with detectable deletions in the lacZ gene were deliberately chosen. The rest of the Mel+ revertants constituted a large number and only a few of them were studied in detail. Each revertant was an independent isolate with unique properties, necessitating the individual description of each revertant. It should be noted that the most frequent class of Mel+ revertants had lost Mu DNA, as determined by Mu immunity tests, and could further revert to Lac+ (108 Mel+ revertant out of 214 isolated behaved in this manner). Clearly, the lesions left in the lacZ gene in these revertants were repairable.

DISCUSSION

This study on Mu DNA excision follows an earlier demonstration that integration of mutator phage Mu can be reversed (Bukhari 1975). The excision of Mu DNA is mostly imprecise; the precise excision is at least 10- to 100-fold less frequent. This phenomenon is particularly significant in view of the general properties of bacteriophage Mu. The phage is a highly promiscuous transposable element and is able to insert its DNA at randomly distributed sites. Yet, from each different site, Mu DNA can be excised, albeit at a low frequency. The excision process of Mu DNA from the host genome parallels the general excision behavior of the transposable elements in bacteria (for discussion of various aspects see Bukhari 1975, 1976; Botstein and Kleckner 1977; Berg 1977; Starlinger 1979; Calos and Miller 1980). The excision of transposable ele-
ments, which is mainly imprecise, is a unique reaction distinct from excision of bacteriophage lambda, in which a site-specific recombination event cleanly excises lambda DNA out of the host chromosome. The mechanisms by which imprecise and precise excisions occur have a bearing on the general problem of how DNA is rearranged.

It is reasonable to assume that precise excision of Mu DNA restores the wild-type host sequences that existed before Mu integration. Thus, removal of Mu DNA from the lacZ gene restores the lacZ+ gene, and the cell reverts to Lac+. However, this observation does not rule out the possibility that one or a few base pair changes can occur at the site from which Mu was excised. These changes would not be detectable if they did not affect the phenotype. This point can be settled only by determining the relevant nucleotide sequences of the Lac+ revertants. In the Lac+ revertants examined, no Mu DNA sequences could be seen by DNA-DNA hybridization. This confirms that Mu DNA is lost from the cells upon excision.

To understand the process of Mu excision, we have concentrated on the ge-

![Figure 12](https://academic.oup.com/genetics/article/98/1/1/5995103)
Figure 13.—Hybridization of the fragments generated by BalI digestion of the DNAs extracted from Mel+ revertants of 8358-X1 with 32P-labeled Mu DNA (autoradiograph after blotting hybridization). (Arrows indicate the presumptive junction fragments.)

netic and biochemical analysis of imprecise excision events. In the system used here (Mu insertions in the lacZ gene causing a polar effect on the Y gene expression), they are detectable as lacZ-Y+ revertants able to express the lacY gene. On the basis of these studies the lacZ-Y+ revertants could be divided into four main categories. The predominant class of the revertants had lost Mu DNA and had no detectable deletions in the Z gene (56%). Most of these Mel+ revertants could revert further to Lac+. These revertants apparently have mutations in the Z gene that can be repaired to the wild-type sequences. A likely possibility is that these are small insertions left behind that can be further excised. In particular, we suggest that a class of these revertants retains 5 base-pair duplications (see CAIOS and MILLER 1980) found at the ends of prophage Mu. These duplications can then be repaired to regenerate wild-type lac. Furthermore, some revertants could have undergone "nearly precise" excision, occurring by recombination between specific sequences within Mu very near the Mu ends, as described by Ross, SWAN and KLECKNER (1979). Such an event would leave a very small specific insertion behind. Sequencing of the lacZ gene of these revertants should resolve this point.

Another class of Mel+ revertants had no detectable Mu DNA and contained deletions of lac sequences. Some deletions spanned both sides of the Mu insertion. However, most of these deletions began from the prophage site and extended to the right or to the left. An important point to note here is that a general class of deletions induced by transposable elements are those in which the element is retained at the site, but the adjacent DNA is deleted. Elements such as IS1, Tn10
and Mu cause these deletions (Reif and Saedler 1975; Toussaint, Faelen and Bukhari 1977; Kleckner, Reichardt and Botstein 1979; Faelen and Toussaint 1980; see for review Calos and Miller 1980; Starlinger 1980). In the case of Mu<sub>cts</sub> X excision, the majority of the deletions cut out the Mu DNA as well. Selection for lac<sub>Y</sub> function in this case demands that Mu DNA be taken out to relieve the polarity caused by Mu insertion. It cannot be ruled out from the present work that those deletions that extended to both sides of the prophage may be spontaneous deletions that occur in E. coli. However, the deletions that extend to only one side of the prophage, particularly the prophages located in the middle of the Z gene, are apparently caused by Mu excision. The relationship between this type of excision and the excision process in which one copy of the element is left behind has yet to be studied.

Surprisingly, in a substantial number of Mel<sup>+</sup> revertants examined (24%), Mu sequences could be detected. In one class of these revertants, there were deletions in the Z gene that presumably extended into the Mu genome, putting the lac<sub>Y</sub> gene under Mu control. In another class, however, the whole Mu genome was still present at the original location in the Z gene. Some of these could be secondary site revertants; for example, unrelated insertions could bring in promoters that could be used to express the Y gene. However, many of the Mel<sup>+</sup> revertants could further revert to Lac<sup>+</sup>, indicating that there was no change in the lacZ gene sequences in the Mel<sup>+</sup> revertants. How can the Y gene be turned on when the whole Mu genome is still present in the Z gene? One must assume that there has been a rearrangement in the Mu sequences and that this rearrangement allows a read-through from Mu into the lac<sub>Y</sub> gene.

It can be seen from the above discussion that many diverse rearrangements are detected when excision of Mu DNA is sought. Some of these rearrangements are probably independent of Mu function since they can occur in the presence of a Muc<sup>+</sup> prophage (where Mu immunity is tightened), or when the Mu<sub>cts</sub> X prophages carry mutations in the A gene of Mu. These incidental rearrangements include particularly those in which Mu sequences are not completely excised. However, a careful analysis of the Mel<sup>+</sup> revertants isolated in the case of Mu<sub>cts</sub> X Aam mutants, or those isolated in the presence of a secondary Muc<sup>+</sup> prophage, to see which rearrangements are entirely host mediated, has not been done. It is clear that a majority (from 75 to 90%) are Mu specific because they require the functioning of the A gene of Mu. If Mu<sub>cts</sub> X Aam mutants are used for excision studies in the Su<sup>-</sup> background, the precise excision is reduced below the detection level and imprecise excision is severely affected.

It seems that transposable elements undergo transposition by a process involving replication of the element such that the element is not excised from the host DNA (Ljungquist and Bukhari 1977; Chaconas, Harshey and Bukhari 1980; see Shapiro 1979; Harshey and Bukhari 1981). This result was somewhat unexpected since excision of Mu DNA had been demonstrated earlier. How is it that a mechanism for Mu DNA excision exists and yet is not operative when Mu DNA is undergoing transposition during the lytic cycle? An answer to this paradox (Khatoon et al. 1979) is inherent to the understanding of mecha-
nisms by which DNA rearrangements occur. It has been proposed that precise excision of an element is merely a host-dependent recombination between duplicated base pairs at each end of the element (see Calos and Miller 1980) and that this recombination per se is not relevant to the process of transposition. (This argument is made for Tn5 by Berg et al. 1980.) In Tn10, Kleckner, Reichardt and Botstein (1979) have shown that the transposon actively participates in events leading to deletions and inversions, but that the exact relationship of these excision-type events to transposition is not clear. In the case of Mu, however, it is clear that the A gene of Mu promotes this excision. The A gene is also required for normal transposition and replication of Mu DNA (Razzak and Bukhari 1975; Fælen, Toussaïnt and Resibois 1978; O’Day, Schultz and Howe 1978; Chaconas, Harshey and Bukhari 1980 and van de Putte et al. 1978). Since the A gene is required for both excision and transposition, we view the excision process as abortive transposition.

The excision of Mu is monitored by using the Mucts X mutants. These are defective prophages that contain mutations, mainly insertions in the B gene of Mu (Khatoon and Bukhari, unpublished), and that cannot kill the cells upon prophage induction. The main block is in the replication of Mu DNA. Thus, the wild-type Mucts prophage (that is, A+ B+) undergoes transposition by replication, but A+ B- derivatives undergo transposition (Fælen, Toussaïnt and Resibois 1978) and excision at a low frequency. It follows, therefore, that the phenomenon of Mu excision is superseded by replication-transposition when A and B (and perhaps other required genes) are functioning normally. If only the A gene is functioning, then the prophage DNA become susceptible to excision.

Perhaps the A gene protein acts at the ends of Mu DNA. If the B gene product and other necessary functions are provided, then the replication-transposition cycle of Mu DNA takes hold. If only the A gene function is present, the prophage is stuck in a state that leads to excision. The excision is a slow and infrequent process since induction of Mucts X mutants (by inactivating the repressor at high temperature) does not lead to an immediate burst of lac+ revertants. It is reasonable to assume that the expression of the A gene is one of the factors determining the frequency of excision. The excision of Mucts X can be seen to some extent even at low temperature, as if the A gene expression is leaky at temperatures where repressor is assumed to be active. The lack of dramatic increase in the excision at high temperature may mean that the production of the A gene is self-limiting. It may be possible to increase the frequency of excision by contriving a situation where A is overproduced. This possibility is being tested. We also suspect that some host DNA synthesis and repair is required for the completion of excision, since in recA- mutants the excision of the Mucts X prophage is severely reduced.

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LITERATURE CITED


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