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Bacteriophage Mu-Induced Modification of DNA Is Dependent upon a Host Function

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The DNA of bacteriophage Mu, extracted from induced lysates, is partially resistant to digestion by the endonuclease *BalI*. This modification of DNA is controlled by the Mu modification function (*mom*), which acts in conjunction with the *dam* (DNA-adenine methylation) function of *Escherichia coli*. Since the *BalI* recognition site is apparently different from the *dam* recognition site, these results imply that either the specificity of the *dam* function is changed by the *mom* function or the *mom* function requires the *dam* function for its activity.

The temperate bacteriophage Mu, grown by induction, is partially insensitive to various restriction systems such as those of bacteriophage P1 and *Escherichia coli* strains B and K (10). This effect is caused by the modification of Mu DNA by a modification function, *mom*, encoded by Mu (10). Allet and Bukhari (2) found that the Mu DNA extracted from induced lysates is not completely digested by the restriction endonuclease *HindII* unless the induced prophage is *mom*. Similar effects have been seen in the case of several other enzymes (R. Kahmann and D. Kamp, personal communication). Toussaint (11) has suggested on the basis of genetic data that the *E. coli* DNA-adenine methylation function (*dam*) is also necessary for Mu DNA modification. In this paper, we present evidence that shows that the cleavage sites for the endonuclease *BalI* are modified on Mu *cts* DNA extracted from induced lysates. This modification, which requires an active *mom* function of Mu and an active *dam* function of *E. coli*, could be clearly seen in the case of Mu *cts X* mutants, carrying one additional *BalI* site.

E. coli strains with the genotypes dam^+ dcm^+, *dam dcm*, and dam^+ *dcm* were lysogenized with Mu *cts*62 (1) or Mu *cts*62 *mom*-3452 (10). Phage lysates were prepared by thermal induction of lysogens according to the method described by Bukhari and Ljungquist (4). The *X* mutants of Mu *cts*62 prophage, located on the F′ *pro lac* episome, were obtained as mutants able to allow reversion of a Mu *cts*-induced mutation as described previously (3). Two independent *X* mutants, 8305-X1 and 8305-X2, were derived from the lysogen BU8305, whereas the *X* mutant 8357-X1 was derived from the lysogen BU8357. Since Mu *cts X* mutants are defective in replication, they were grown with the help of a normal Mu *cts* prophage, after being conjugally transferred (with F′ *pro lac*) to a lysogen. DNA from phage lysates was obtained according to the earlier described method (4) and digested with the endonuclease *BalI* (from *Brevibacterium albidum*) or with *BalI* and *EcoRI* (an endonuclease from *E. coli* RY13). After digestion of DNA according to the method of Sharp et al. (9), the restriction fragments were resolved by electrophoresis (9) on 1% agarose gel, containing ethidium bromide (1 μg/ml), and photographed under UV light.

The *BalI* digestion patterns of various Mu *cts* DNA preparations are shown in Fig. 1A. It can be seen that *BalI* cleaves Mu *cts* DNA at three sites (i, ii, and iii) to generate four fragments, a, b, c, and d, of which a and b constitute the internal fragments and c and d constitute the end fragments. The sizes of these fragments are estimated to be approximately 22, 11, 4.5, and 1.2 kilobases, respectively (7). The end fragments, c and d, appear fuzzy because of the presence of host DNA sequences of heterogeneous lengths. In the case of Mu *cts* mom^+ DNA, originating from the induced lysates of dam^+ cells (Fig. 1A, slots 1 and 2), the majority of the DNA remained uncut. However, when DNA originated from mom^+ phage grown on *dam* cells or from *mom* phage grown on dam^+ cells (or *dam* cells), it was cleaved to yield sharply defined bands (Fig. 1A, slots 3, 4, 5, and 6). The intensity of band b, in particular, was greatly enhanced. The effect of modification on recovery of specific bands appears to correlate with the number of *BalI* cleavages needed to produce them. It is greater for band b, requiring two cleavages, and less for bands c and d, requiring only one cleavage each. Since band a is not clearly resolved from uncut DNA, the effect of

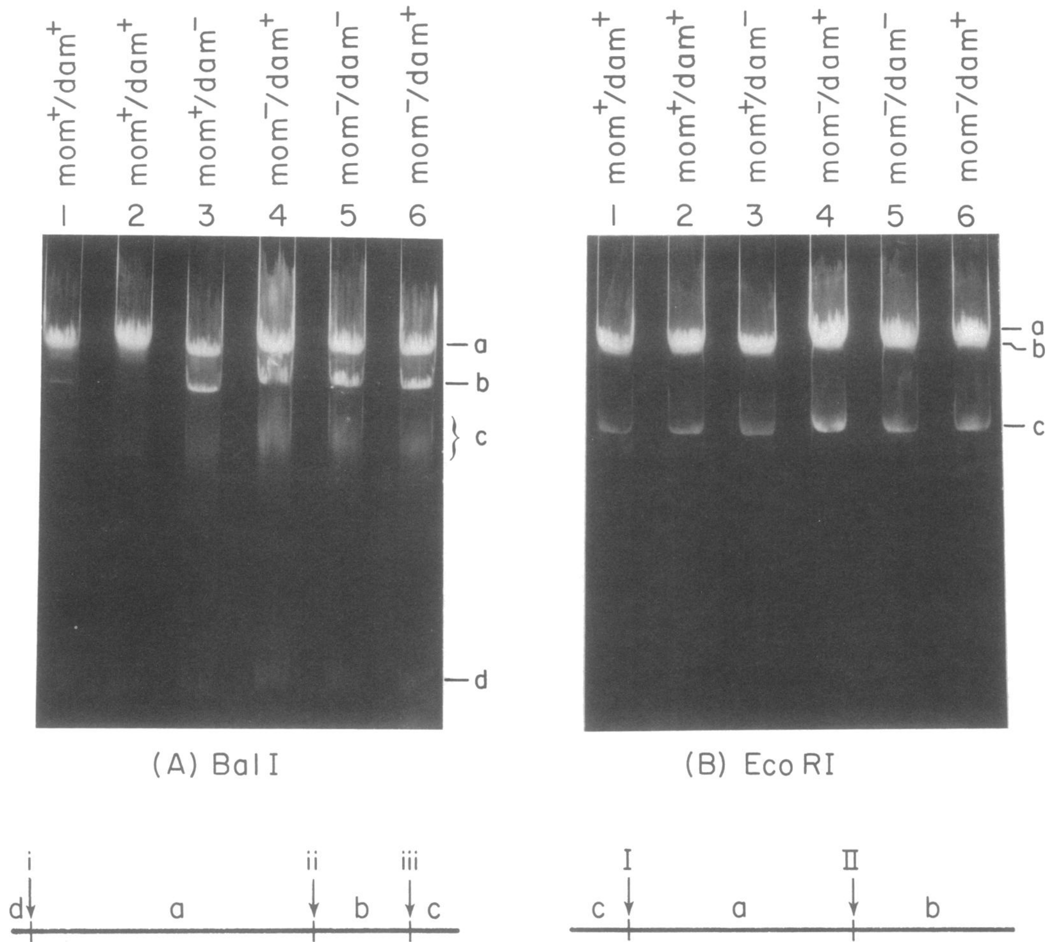
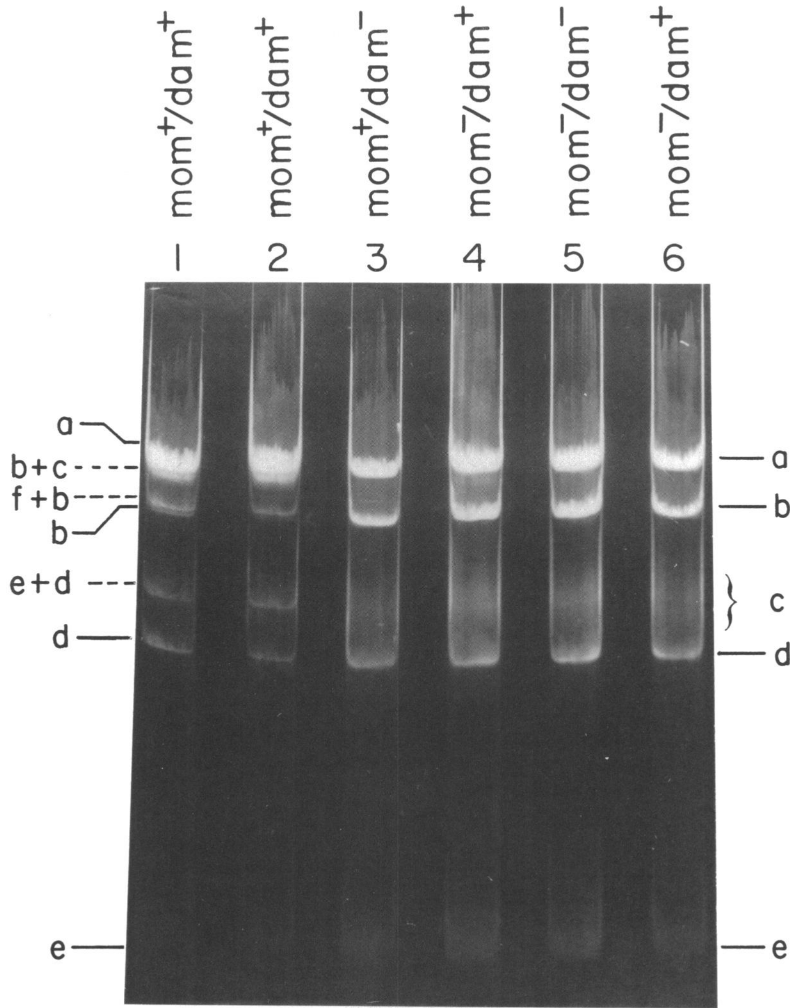


FIG. 1. *Agarose gel electrophoresis of Mu cts DNA fragments after digestion (A) with the endonuclease BalI and (B) with the endonuclease EcoRI. All the DNAs were extracted from Mu cts particles obtained by induction of E. coli lysogens. Slot 1, Mu cts mom$^+$ DNA from the lysates of BU8305 (dam$^+$ dcm$^+$); slot 2, Mu cts mom$^+$ DNA from BU1616 (dam$^+$ dcm); slot 3, Mu cts mom$^+$ DNA from BU1617 (dam dcm); slot 4, Mu cts mom DNA from BU1618 (dam$^+$ dcm); slot 5, Mu cts mom DNA from BU1619 (dam dcm); slot 6, Mu cts mom DNA from BU165 (dam$^+$ dcm$^+$). The numbers i, ii, and iii refer to BalI cleavage sites; I and II refer to EcoRI cleavage sites on Mu cts DNA. a, b, c, and d represent the cleavage fragments.*

modification on its generation cannot be determined. From these results, it is apparent that both *mom* and *dam* functions are needed for the modification of *BalI* sites on Mu DNA.

A further confirmation of the modification of *BalI* sites comes from the double digestion of Mu DNA with *BalI* and *EcoRI*. The endonuclease *EcoRI* cleaves Mu DNA equally well whether it is modified or not (2; Fig. 1B). All the DNAs studied for *BalI* digestion (Fig. 1A) were equally well digested with *EcoRI* (Fig. 1B). The *EcoRI* sites (I and II, Fig. 1B and Fig. 2) are distributed in such a way on Mu DNA that we can monitor the modification of *BalI* sites i and ii by using a combination of *BalI* and *EcoRI*. When we performed the double digestion, the results were very similar to those obtained with *BalI* digestion alone (Fig. 2). All *BalI* sites were properly cleaved when DNA originated from a *mom* phage grown on a *dam*$^+$ or a *dam* host (Fig. 2, slots 4, 5, and 6) or from a *mom*$^+$ phage obtained from a *dam* host (slot 3, Fig. 2). Sharp bands a, b, c, d, and e were observed as expected from normal cleavage of all sites. The sixth fragment, f, was lost from the gel in Fig. 2 because of its small size. When Mu *mom*$^+$ DNAs obtained from the lysates of *dam*$^+$ cells were digested with *BalI* plus *EcoRI*, several addi-



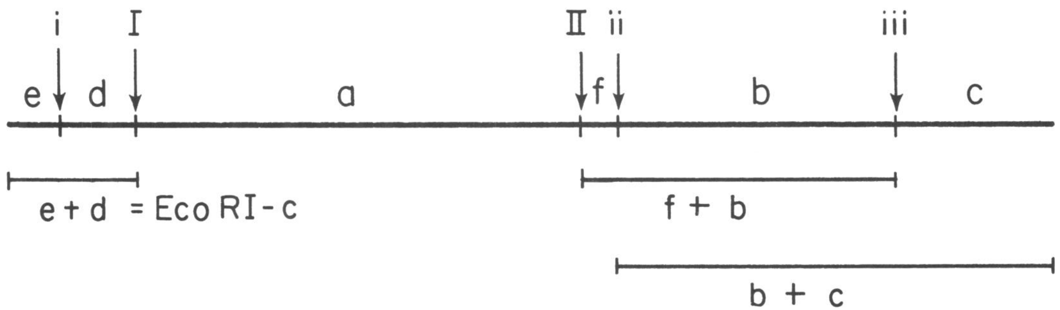


FIG. 2. *Agarose gel electrophoresis of Mu cts DNA fragments generated by double digestion with the endonucleases BalI and EcoRI. All the DNAs were extracted from Mu cts particles grown by induction of E. coli lysogens. Slot 1, Mu cts mom⁺ DNA from the lysates of BU8305 (dam⁺ dcm⁺); slot 2, Mu cts mom⁺ DNA from BU1616 (dam⁺ dcm); slot 3, Mu cts mom⁺ DNA from BU1617 (dam dcm); slot 4, Mu cts mom DNA from BU1618 (dam⁺ dcm); slot 5, Mu cts mom DNA from BU1619 (dam dcm); slot 6, Mu cts mom DNA from BU165 (dam⁺ dcm⁺). The numbers i, ii, and iii refer to BalI cleavage sites; I and II refer to EcoRI cleavage sites on Mu cts DNA. a, b, c, d, e, f, etc., represent the cleavage fragments.*

tional bands were observed (slots 1 and 2, Fig. 2). The band e + d represented the original *Eco*RI band c (Fig. 1B), which remained uncleaved because of the modification of *Bal*I site i in some DNA molecules. The band f + b apparently arose from the partial modification of *Bal*I site ii, and the band b + c arose from the partial modification of *Bal*I site iii. The band b + c could also be the original *Eco*RI band b (Fig. 1B), which remained uncleaved because of the modification of *Bal*I sites ii and iii.

Modification of the *Bal*I site i was more

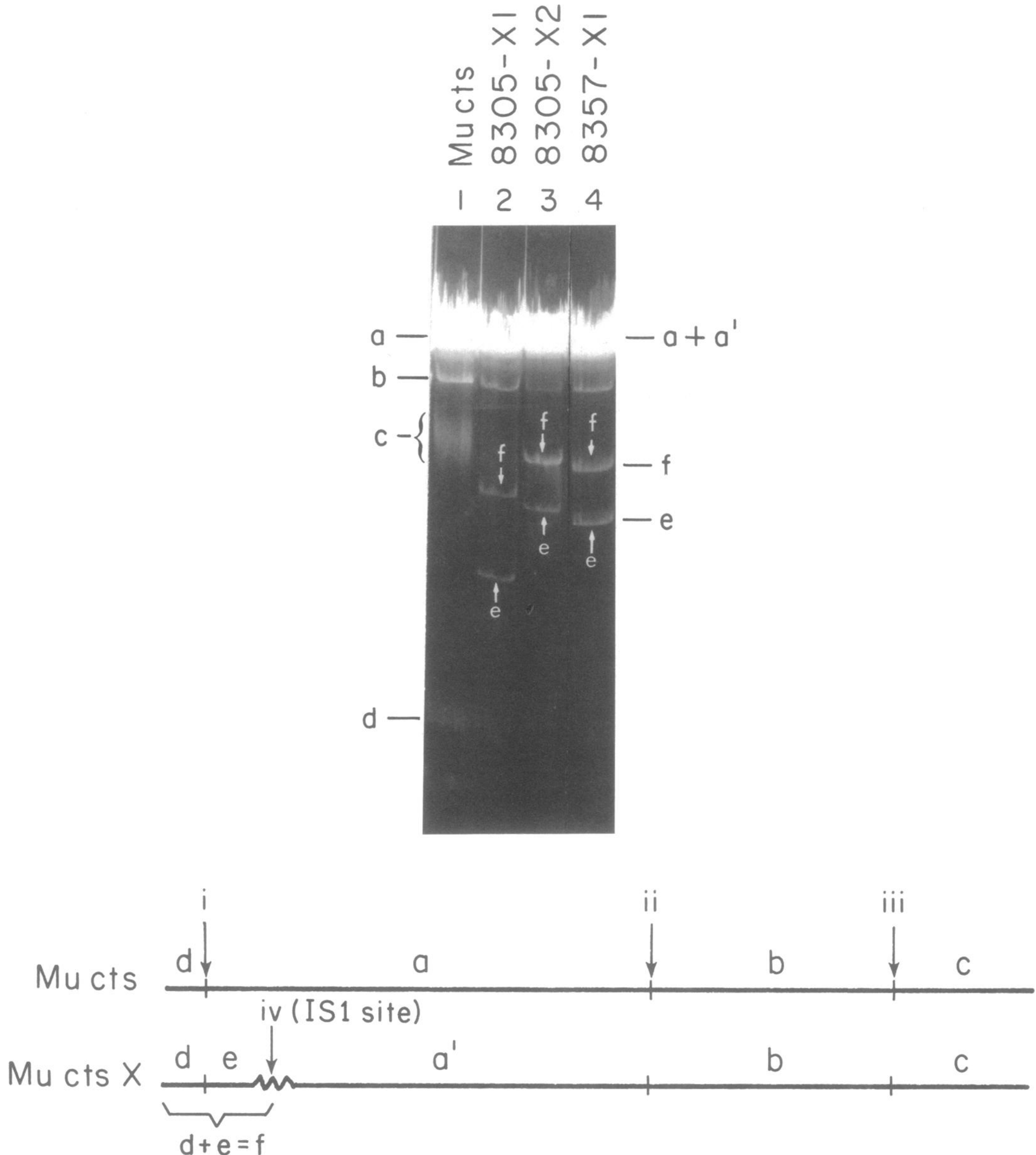


FIG. 3. *Agarose gel electrophoresis of the fragments generated by BalI cleavage of Mu cts and Mu cts X DNAs. All the DNAs were obtained from induced lysates of dam⁺ cells. Slot 1, Mu cts DNA from BU8305; slot 2, DNA of Mu cts X mutant 8305-X1; slot 3, DNA of Mu cts X mutant 8305-X2; slot 4, DNA of Mu cts X mutant 8357-X1. All the X mutants were grown with Mu cts helper phage. The numbers i, ii, iii, and iv refer to BalI cleavage sites on Mu cts and Mu cts X DNAs. The jagged line represents the insertion of IS1 element in Mu cts X DNA. a, a', b, c, d, e, and f represent the DNA fragments generated after cleavage with BalI.*

clearly observable when we used Mu *cts X* DNA, possessing one extra *Bal*I site (site iv, Fig. 3), imparted by IS*1* insertion (5). We expected that cleavage of Mu *cts X* DNA with *Bal*I would result in the generation of a new small fragment (fragment e, Fig. 3) and a new large fragment a′, which would not be resolved from fragment a because of its size. *Bal*I digestion of DNA from *X* mutant lysates grown in dam^+ cells with a Mu *cts* mom^+ helper phage produced two extra small fragments, e and f (Fig. 3). The size of fragment e varies from one *X* mutant to another, depending upon the distance of site iv from site i. By examining fragments e and f arising from different *X* mutants (Fig. 3), we found that the size of fragment f depended upon the size of fragment e in such a way that any decrease in the size of fragment e also resulted in a decrease in the size of fragment f and vice versa (Fig. 3, slots 2, 3, and 4). Considering the possibility that f arose because of the partial modification of site i, we transferred the *X* mutants 8305-X1 and 8357-X1 to the *dam* strains (BU1646 and BU1645, respectively) and cultivated them with the help of a Mu *cts mom* (*mom*-3452) helper phage. DNA extracted from the double lysates was digested with *Bal*I, and fragments were separated by gel electrophoresis (Fig. 4). By comparing the restriction patterns of 8305-X1 and 8357-X1 in dam^+ and *dam* cells, it becomes obvious that fragment f disappears in both cases when Mu *cts X* DNA is obtained from the lysates of *dam* cells (Fig. 4). This finding, in addition to the finding that bands e and f are almost equal in intensity (Fig. 3, slots 2, 3, and 4; Fig. 4, slots 2 and 4), provides sufficient proof that *Bal*I site i is modified in about half of the DNA molecules.

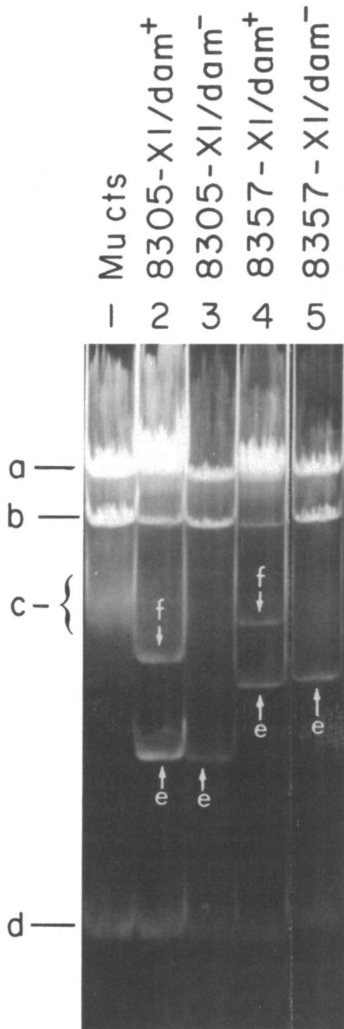


FIG. 4. *Agarose gel electrophoresis of the fragments generated by BalI cleavage of Mu cts and Mu cts X DNAs. All the DNAs were obtained from induced lysates of E. coli lysogens. Slot 1, Mu cts (mom) DNA from the lysates of BU1619 (dam dcm); slot 2, DNA of Mu cts X mutant 8305-X1 from the lysates of BU1570 (dam⁺ dcm⁺); slot 3, DNA of Mu cts X mutant 8305-X1 from the lysates of BU1646 (dam dcm); slot 4, DNA of Mu cts X mutant 8357-X1 from the lysates of BU1566 (dam⁺ dcm⁺); slot 5, DNA of Mu cts X mutant 8357-X1 from the lysates of BU1645 (dam dcm). a, b, c, d, e, and f represent cleavage fragments.*

From the results presented above, it is clear that both the *mom* function of the phage and the *dam* function of the host are necessary for Mu DNA modification. Whether or not all of the *Bal*I sites are modified to the same extent is still not clear.

The apparently concerted action of the Mu *mom* function and the *E. coli dam* function provides a highly interesting example of a virus-host interaction affecting modification of DNA. The nature of the *mom-dam* interaction is intriguing. *Bal*I recognizes the hexanucleotide 5′-TGGCCA-3′ and cuts it in the middle (6). The *dam* function itself clearly cannot modify this sequence and, in fact, modifies the adenine residue in the sequence 5′-GATC-3′ (8). It must follow, therefore, that either the specificity of the *dam* function is changed by the *mom* function or the *mom* function requires the *dam* function for its activity.

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