Temperature-Sensitive Mutants of Bacteriophage Mu

A. I. BUKHARI AND PAULA CURTIN

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Received for publication 23 August 1974

Temperature-sensitive mutants of bacteriophage Mu, which grow at 32 C but not at 42 C, have been isolated. These mutants fall into two groups. Group 1 mutants fail to lyse host cells at nonpermissive temperatures, whereas lysis occurs normally with the group 2 mutants. All of the group 1 mutants apparently belong to the cistrons mapping to the left of gene C, whereas the group 2 mutants have lesions in various genes between D and S.

The temperate bacteriophage Mu (5) can seemingly enter the DNA of *Escherichia coli* at random points to establish lysogeny (3). Temperature-sensitive (*ts*) mutants of Mu would be helpful in elucidating many aspects of the Mu life cycle. We report here the isolation of mutants which are blocked in either early or late stages of Mu growth at nonpermissive temperatures.

The mutants were obtained by *N*-methyl-*N'*-nitro-nitrosoguanidine (NTG) mutagenesis of Mucts62, a heat-inducible derivative of Mu-1. Mucts62 makes clear plaques at 42 C but turbid plaques at 32 C, owing to a mutation in the immunity gene C (1, 4). An *E. coli* K12 strain lysogenic for Mucts62, BU8305, was used for mutagenesis. BU8305 carries an F' *pro*⁺ *lac* episome with Mucts62 inserted into the *lacZ* gene and has the genotype F' *pro*⁺ *lacZ*: (Mucts62)/Δ *pro lac*, *his*⁻, *met*⁻ (2). Cultures of BU8305 were grown at 32 C in L-broth, containing tryptone and yeast extract (2), to mid-log phase, centrifuged, and resuspended in 0.2 M sodium acetate, pH 5.0, with 30 μg of NTG per ml. After 1 to 2 h at room temperature, the cells were washed to remove NTG and the survivors were grown to saturation in L-broth. The mutagenized cultures were plated on minimal medium supplemented with histidine and methionine (2) to obtain about 200 to 400 colonies per plate. The colonies were replica plated onto lawns of Mu-sensitive bacteria in 0.7% soft agar on L-agar plates, and the plates were incubated at 32 and 42 C. Those colonies which produced phage at 32 C, as indicated by the killing of sensitive bacteria around the colonies, but not at 42 C, were purified. The frequency of such mutant clones was less than 5 × 10⁻³. To determine whether the mutant clones carried mutations in the Mu genome, phage particles were isolated from cultures grown at 32 C and tested for plaque-forming ability at 32 and 42 C.

TABLE 1. Characterization of the *ts* mutants

Mutant no.	Complementation group ^a	Host-cell lysis at 43 C
5045	A	—
5074	A	—
5038	B or Bu	—
5051	B or Bu	—
5087	B or Bu	—
5037	D	+
5062	D	+
5063	D	+
5100	J	+
5017	L	+
5048	L	+
5093	L	+
5075	M	+
5042	N	+
5149	S	+

^a Complementation tests with Mu amber mutants were done by mixing 10⁷ to 10⁸ amber particles with 0.1 ml of a culture of strain 40 (F⁻ Su⁻, ref. 2) on L-agar plates and spotting the *ts* mutants on the lawn. The tests were also done by mixing the *ts* mutants in the lawn and spotting the amber mutants. The plates were incubated at 43 C, and extensive lysis in the spots, as compared to the controls, was taken to indicate complementation. A *ts* mutant was assigned to a cistron if an amber mutant of that cistron did not complement the *ts* mutant at 43 C. The Mu amber mutants used (lysates obtained from M. Howe) were Aam1093, Bam1066, Buam5151, Buam5152, Cam4105, lysam1025, Dam3801, Eam1006, Fam1065, Gam1042, Ham1043, Iam4037, Jam1155, Kam1010, Lam1007, Mam1114, Nam1041, Oam1133, Pam1024, Qam1074, Kam1059, and Sam1004. Complementation groups for four other *ts* mutants (5073, probably D; 5083, probably B; 5085 and 5086, probably A) have not been clearly established. Lysis of host cells at 43 C was determined by monitoring turbidity in the Klett-Summerson colorimeter.

Nineteen phage mutants which could be propagated at 32 C but not at 42 C were charac-

FIG. 1. Growth response of *ts* lysogens after induction. The cultures were grown in *L*-broth by shaking at 32°C, and shifted to 43°C at the times pointed by the arrows. The lysogens are indicated by the number of the *ts* mutants they contain. *Mu*cts62 represents the parent lysogen. A heat-pulse experiment in which the culture was shifted to 43°C for 30 min and then brought back to 32°C is indicated by 42 → 32. The turbidity of the cultures was followed in a Klett-Summerson colorimeter with a no. 66 filter (640 to 670 mμ).

FIG. 2. Genetic map of *Mu*. The map shown, with genes arranged in alphabetical order, is the cumulative Cold Spring Harbor map (1). *Bu* is a tentative new cistron, which is adjacent to gene *B*, between *A* and *B* (A. Bukhari and M. Howe, unpublished data). Stars indicate the genes to which the *ts* mutants have been assigned so far.

terized. Table 1 shows the *Mu* cistrons to which lesions causing temperature sensitivity were assigned on the basis of complementation by amber mutants of all the known essential genes of *Mu*. As can be seen from the table, the mutants fell into two groups. Group 1 mutants did not cause significant lysis of the host cells at the nonpermissive temperature, whereas lysis occurred normally with the group 2 mutants. Figure 1 shows the typical growth response of the lysogenic cells, containing representative

mutants of the two groups, after prophage induction. The *Nts*5042 lysogenic cells lysed rapidly about 1 h after being shifted to 43°C. In the case of *Ats*5045 no rapid lysis occurred although the growth of the culture was reduced markedly, eventually coming to a halt. However, when the culture was shifted to 32°C after a 30-min heat pulse at 43°C, it appeared to lyse normally.

The mutants of group 1 were not merely defective in rupturing host cells at the nonpermissive temperature, but their burst sizes, determined by opening the cells with chloroform, were at least 10^3 -fold lower than *Mu*cts62 at 42°C. This suggests that they have temperature-sensitive blocks in the early *Mu* functions which are necessary for the late stages of the lytic cycle, including the expression of the lysis function. These mutants belonged exclusively to the A-Bu-B region, which is located to the left of gene *C* on the genetic map of *Mu* shown in Fig. 2. Apparently, the genes to the left of gene *C* constitute the "early" region of the genome. The genes *A* and *B* are known to be involved in *Mu* integration and DNA replication (6). The gene *A* mutants are particularly interesting since they appear to be blocked in the excision of *Mu* DNA as monitored by the reversion of *Mu*-induced mutations (A. Bukhari, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, p. 218; manuscript in preparation). The *ts* mutants of *Mu* are being used to further study the integration, excision, and replication of *Mu* DNA.

This work was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research and the National Science Foundation to A.I.B. (CB-43280) and by Public Health Service grant GM17612-04 from the National Institute of General Medical Sciences to D. Zipser.

LITERATURE CITED

1. Abelson, J., W. Boram, A. I. Bukhari, M. Faalen, M. Howe, M. Metlay, A. L. Taylor, A. Toussaint, P. van de Putte, G. C. Westmaas, and C. A. Wijffelman. 1973. Summary of the genetic mapping of prophage *Mu*. *Virology* 54:90-92.
2. Bukhari, A. I., and M. Metlay. 1973. Genetic mapping of prophage *Mu*. *Virology* 54:109-116.
3. Bukhari, A. I., and D. Zipser. 1972. Random insertion of *Mu*-1 DNA within a single gene. *Nature N. Biol.* 236:240-243.
4. Howe, M. M. 1973. Prophage deletion mapping of bacteriophage *Mu*-1. *Virology* 54:93-101.
5. Taylor, A. L. 1963. Bacteriophage-induced mutation in *Escherichia coli*. *Proc. Nat. Acad. Sci. U.S.A.* 50:1043-1051.
6. 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.