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THE MECHANISM OF INTEGRATION OF PHAGE Mu IN THE CHROMOSOME OF ESCHERICHIA COLI

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SUMMARY. Cultures of synchronized E. coli were infected with phage Mu at various stages of the division cycle. Phage integration at a given locus on the chromosome was measured by the lost of the corresponding gene function. For several loci, maximal integration occured at gene locus during replication of that locus.

Phage Mu inactivates the genes within which it integrates (1). The insertion appears to occur at random along the chromosome (2). It has been proposed that this phage integrates preferentially in the replicating fork (3). This assumption is based on the observation that in bacteria treated with nalidixic acid (which promotes replication at the chromosome origin) subsequent infection with Mu preferentially caused mutations within genes near the chromosome origin. The magnitude of the effect was greater than what could be accounted for by a mere gene dosage. In the present work, this view has been substantiated by the finding that, in synchronized cultures, Mu induced mutation at a given locus is maximal if infection takes place when replication is in progres in the corresponding region.

MATERIAL AND METHODS. The bacterial strain utilized is E. coli K12 R199 lac:: (Mu cts62 X) harbouring an excision defective Mu prophage with a thermosensitive repressor. The repression activity is lost at 42°C and is recovered in about 70 per cent of the bacterial population within 15 min. following a temperature shift to 32°C (fig. 1).

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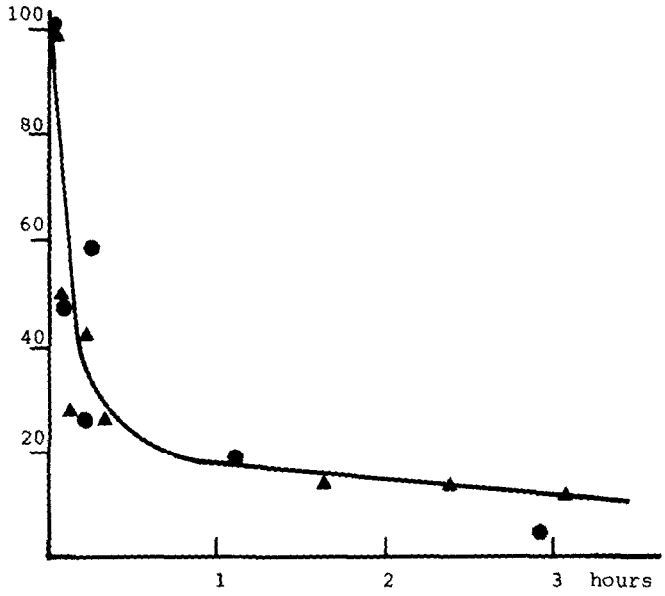

Figure 1. Kinetics of recovery of lysogenic immunity. Culture of E. coli K12 R199 lac:: (Mu cts62 X) were incubated at 42°C for 10 generations; the temperature was then shifted down to 32°C. Aliquots were taken at various times, infected with 1 Mu phage for ten cells. After 6 min. incubation, the non-adsorbed phages were eliminated by centrifugation and the infected cells were tested on C600 strain for the ability to release phages. Ordinates : % infective centers. Abscissae : time.

The growth medium is LB (4) supplemented with $CaCl_2$:2,5.10^{-3}M and $MgSO_4$:10^{-3}M for phage adsorption. The low phosphate medium used for synchronization has the following composition:Tris:0,1M; NaCl:0,1 per cent; Glucose:0,4 per cent; $CaCl_2$:10^{-4}M; $MgSO_4$:10^{-3}M; Casaminoacid Difco:0,08 per cent, containing 10^{-4}M of inorganic phosphate.

Synchronization is achieved following a method developed by Kepes and al (unpublished) based on an observation of Goodwin (5) according to which phosphate pulses on a culture in a chemostat synchronize cell division. In our conditions, the cells are grown in a chemostat and subjected to eight successive 3 min. pulses of low phosphate medium at 90 minute intervals. At each addition of medium the suspension is diluted twofold and recovers subsequently

its original density within 50 minutes, then stops owing to the limited amount of phosphate. Thereafter, the cells are washed, suspended, (2.10^7 cells/ml) in LB medium and incubated at 42°C where they divide synchronously for 3 to 4 generations.

Infection with Muc+ is performed at various stages of the synchronized culture at 42°C. Every 5 min. 1 ml samples of the culture are withdrawn and infected with Mu (at a m.o.i. of 7 phages/cell) at 42°C for 8 min. After infection the cells are shifted to 32°C; this procedure insures that most integrations occured at 42°C (6). Then the samples are diluted 1/100 in LB medium and allowed to grow for 18 hours at 32°C with aeration before scoring for the various mutants (see legend of fig. 3).

It is assumed that the growth rate of the bacteria with different phenotypes is the same in LB medium at 32°C.

RESULTS AND DISCUSSION.- Figure 1 describes the kinetics of the shift from the non immune to the immune phase in the R199. This strain was obtained from among the survivors of thermal induction of RS54 lac:: (Mu cts62). Approximately 2 per cent of the survivors screened in this manner have the phenotype described in fig. 1, whereas the others don't recover immunity at 32°C. This mutant should be compared to the one described by FAELEN et al. (7). In addition, as R199 complements with amber mutants of A and B genes of Mu, it is possible that this strain behaves like a kil$^-$ mutant (8) which restores immunity at 32°C.

In figure 2 are given the variations in the colony counts and the kinetics of DNA synthesis in the bacterial population after its transfer from the chemostat to the non limited phosphate medium. There is a lag of about 20 min. in the start of DNA synthesis. It has consistantly been observed that the first cell division takes a substancially longer time than the subsequent

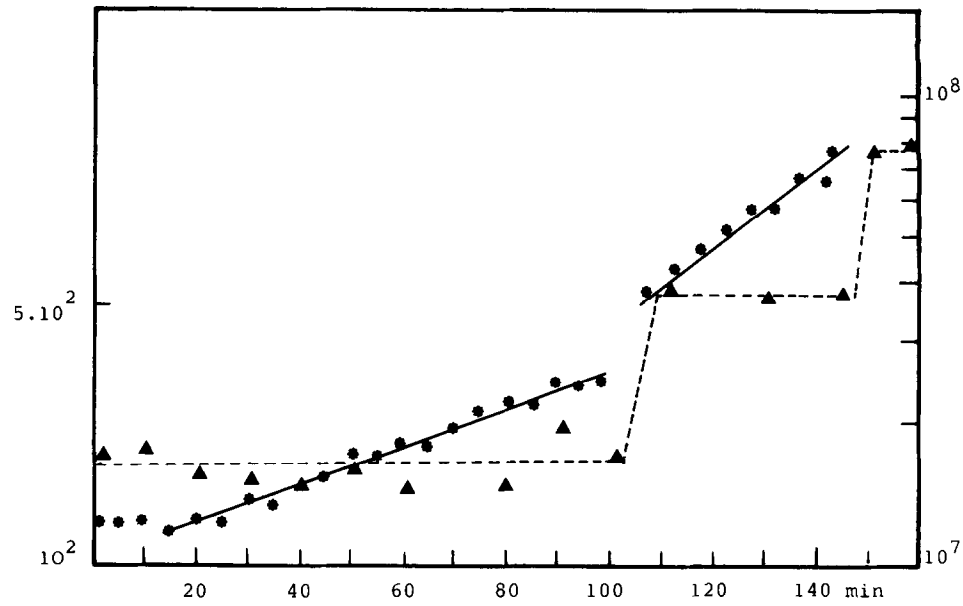

Figure 2. Synchronization. Synchronized bacteria were incubated in LB medium as described in the text and aliquots were taken at various times. At t_0 culture was labelled with ^{14}C guanine (present at 0.2 uCi/ml, 4 ug/ml). The colony forming bacteria (●) and the incorporation of ^{14}C guanine in alkali resistant precipitable counts were determined (▲). Ordinates : left - cp.m. : right colonies forming bacteria. Abscissae : time.

ones. In this culture, the lysogenization frequency among the survivors after infection was determined at 20, 40 and 60 min. and found to the respectively 6.10^{-2} ; 7.10^{-2} ; 7.10^{-2}.

In figure 3 are given the frequencies of induced mutants for the characters xyl, malA, malB and gal, the location of which is represented in the diagram of the genetic map. For each character a maximum is observed for the mutation frequency after which there is a substantial decrease. The maximum is above the level of the induced mutation frequency for the corresponding marker in Mu infected non synchronized cultures.

The random integration of Mu in a synchronized population of bacteria would show different kinetics according to the location of the gene concerned with regard to the origin of replication.

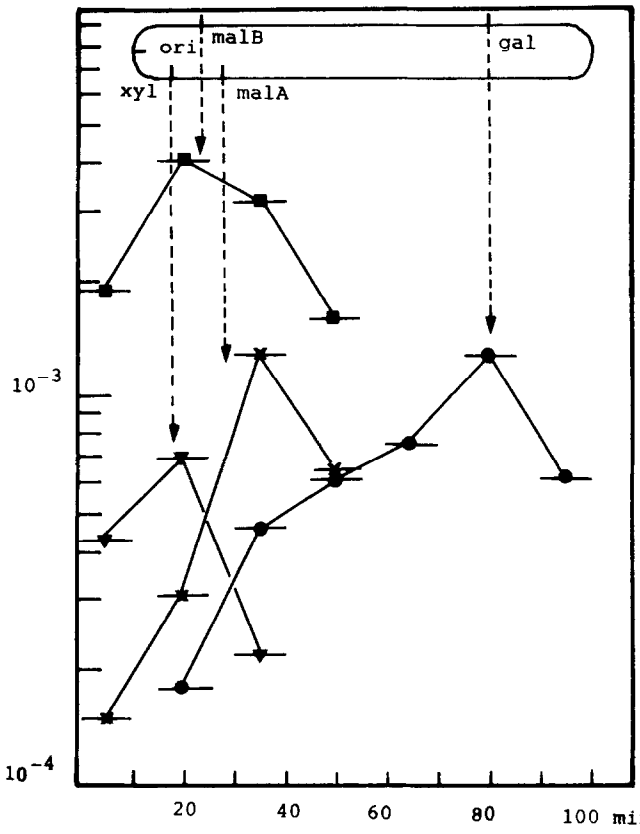

Figure 3. Distribution of mutants among lysogens in synchronized culture. Samples from the culture Fig.1 were taken every 5 minutes and infected at 42°C in LB medium at a multiplicity of 7 phages per cell. The samples were kept for 8 minutes at 42°C before being diluted 1/100 in LB medium and incubated for 18 hours at 32°C. The samples were pooled three by three (0-5-10; 15-20-25;..min.) and the frequency of the mutants unable to utilise the various sugars is determined on Mc Conkey medium. The clones were tested for immunity to Mu. The malA were controled to be also resistant to lambda vir phages. Lambda sensitive mal mutants were further characterized by mating them with F' strain KLF11/JC1533, which carries the malB marker. Those mutants which recover a mal^+ phenotype were considered mutated in the malB region.
Ordinates frequency of the various mutants. The horizontal bars correspond to the grouping by three of the samples. malA ✖ , malB ■ xyl ▼ , gal ● Abscissae : time in minutes.
At the upper part of the figure is schematized the chromosome with the location of the various markers with respect to the progression of the replicating process during the division cycle.
Ordinates : mutant frequency. Abscissae : time in minutes.

For the proximal markers a maximum, at the moment of gene doubling, would be expected, followed by a regular decrease. For the distal markers a continuous decrease would be observed until gene replica-

tion, when a sudden increase would be expected.

This is obvioulsy in contradiction with the mutation kinetics obtained for the gal and malA markers.

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