INHIBITION OF DNA SYNTHESIS AND INDUCTION OF LAMBDA PHAGE IN TEMPERATURE SENSITIVE MUTANTS OF *ESCHERICHIA COLI*¹⁾

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The prophage λ of *Escherichia coli* K-12 can be induced by a variety of treatments which have the common feature of inhibiting DNA synthesis. (Weigle and Delbrück 1951, Otsuji *et al.* 1959, Korn and Weissbach 1962). We isolated mutants of *E. coli* K-12, DNA synthesis of which is blocked at a high temperature. This paper describes the results which indicate that λ lysogenized in these mutants can be induced at a high temperature.

The temperature sensitive mutants were isolated from a non-lysogenic thymineless F⁻ strain. The experiments reported here were performed mostly with one of such mutants, ST8. DNA synthesis of this mutant ceased after about 1.5 hours upon transfer from 30° to 42° (Fig. 1), but RNA or protein synthesis continued for several hours. Assays of activities at 42° and 30° of DNA polymerase and exonucleases I and III with extracts of this strain grown at 30° indicate that the activities of these enzymes are not temperature sensitive.

While the viable count of ST8(λ)⁻ (non-lysogenic strain) continued to increase for about 2 hours after transfer to 42°, that of ST8 (λ)⁺ (ST8 strain lysogenized with λ) decreased rapidly under the same conditions (Fig. 2). This observation suggests that ST8(λ)⁺ strain was killed at 42° as the consequence of induction of λ phage. But production of mature phages was not detected by plaque counting. As is shown in Figs. 3 and 4, however, if the culture was incubated at 30° following incubation at 42°, production of mature λ phages was observed. These results are interpreted to indicate that the initial step(s) for prophage induction occurs during the incubation at 42°, but further growth of the phage is arrested at a certain stage. Further development of the phage can take place only at a lower temperature, e. g. 30°.

Preliminary experiments with 5 other bacterial mutants, DNA synthesis of which was similarly blocked at 42°, showed that λ phage was induced by incubation at 42° in all of them but the synthesis of mature phage could not proceed unless the treated cultures were returned to 30°.

The results presented in this paper support the idea that prophage induction is a general consequence of inhibition of host DNA synthesis.

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Fig. 1 Incorporation of ^{14}C -thymine in acid-insoluble fraction of $\text{ST8}(\lambda)^-$ strain at 30° and 42° . The medium contained glucose, amino acids, thymine and mineral salts. The ^{14}C -thymine was added to the culture at the time indicated by the arrow. At time 0, the culture was divided into two portions; one was transferred to 42° (●; CPM, ▲; Optical density), while the other was kept at 30° (○; CPM, △; Optical density). The quantity of DNA synthesised at 42° was calculated to be about 80% of the DNA present at the time of transfer to 42° .

Fig. 2 Cultures of $\text{ST8}(\lambda)^-$ and $\text{ST8}(\lambda)^+$ were grown for 2 hours at 30° . Each culture was then divided into 2 portions, one of which was transferred to 42° , while the other was kept at 30° . Colony forming cells were determined at intervals by plating on nutrient agar, which was incubated at 30° .

Fig. 3 $\text{ST8}(\lambda)^+$ culture was transferred from 30° to 42° at time 0. Samples were withdrawn at intervals, returned to 30° , and incubated for 2 hours. The samples were lysed by addition of chloroform. The number of λ determined by plating with indicator bacteria is plotted against the time of transfer from 42° to 30° .

Fig. 4 For this experiment a thy^+ revertant of $\text{ST8}(\lambda)^+$ was used and thymine was omitted from the medium. The culture was transferred from 30° to 42° and incubated for 3 hours. Then it was returned to 30° . At intervals, samples were withdrawn for determination of plaque forming units. Number of total cells was counted by microscopy, and that of viable cells was determined by plating at 30° .

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