Bacteriophage Lysozyme Synthesis in *Escherichia coli* K-12(λ) Infected with rII Mutant of Bacteriophage T4

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When *Escherichia coli* K-12(λ) was infected with a T4rII bacteriophage, synthesis of lysozyme appeared at the normal time but stopped 15 min after infection. The lysozyme produced was 1% of the normal level.

The genetic structure of the rII gene of bacteriophage T4 has been carefully studied (2), but the physiology of the rII gene is still largely unknown (3-5, 8, 10, 11). In this respect, B. Rutberg and L. Rutberg (9) reported that, when *Escherichia coli* K(λ) cells were infected with rII mutants of phage T4, deoxycytidine triphosphatase was produced at the same rate as in r^+ -infected cells. Deoxyribonuclease activity was about one-third to one-half of that of r^+ -infected cells, but no lysozyme activity was detected in rII-infected K(λ) cells. Other reports show that the initiation of deoxyribonucleic acid (DNA) synthesis is normal in rII-infected K-12(λ), but DNA synthesis is blocked at a later stage (4, 5, 8). In a permissive host, phage lysozyme synthesis starts at about the same time as the initiation of DNA synthesis (7). We might expect, therefore, that some lysozyme was synthesized before termination of DNA synthesis, but at levels too low to be detected by the assay method used by Rutberg and Rutberg (9). I have used a more sensitive assay (6) to re-examine phage lysozyme synthesis in T4rII-infected *E. coli* K-12(λ).

K-12(λ) cells from an overnight culture were diluted 100-fold into tryptone broth (7) and grown with aeration at 37°C for about 4 hr until the titer reached 5×10^8 cells/ml. The cell titer was determined by phase-contrast microscope counting and by plating (1). The culture was then chilled rapidly by aerating in an ice bath. The cells were centrifuged in a Sorvall refrigerated centrifuge at 5,000 rev/min for 10 min. The cells were immediately resuspended in cold tryptone broth and adjusted to a concentration of 5×10^8 cells/ml. The cells were then aerated at 37°C for a total of 10 min prior to infection in a tube which allowed sampling without interruption of aeration. The bacterial cells were infected with T4D or

T4DrII187 mutant at a multiplicity of five phage per bacterium. At various times, 5-ml samples were transferred to precooled vials and quickly

FIG. 1. Phage lysozyme activity in *Escherichia coli* K-12(λ) infected with T4 r^+ (○) or T4rII (●) mutant.

frozen in a dry ice-acetone mixture. These frozen samples were thawed one by one just before rupturing the cells by ultrasonic treatment. After ultrasonic treatment (7), the samples were kept at

0°C until the lysozyme assay was performed. The lysozyme assay method used for this study was described by K. K. Mark (6).

It was observed that cells infected with wild-type T4 started to synthesize phage lysozyme at about 8 min after infection. The level of lysozyme increased linearly up to about 30 min after infection, at which time the lysozyme activity in the ultrasonically treated sample reached a level equivalent to 0.023 mg of egg white lysozyme per ml. The T4rII mutant-infected cells started to synthesize phage lysozyme at about the same time (8 min after infection), but the level, as shown in Fig. 1, was very low. Such lysozyme synthesis was stopped at approximately 15 min after infection. The final lysozyme level at 25 min after infection was equivalent to 0.0002 mg of egg white lysozyme per ml, about 1% of the wild-type level.

The fact that lysozyme synthesis and DNA replication stopped at the same time may be related to the requirement for DNA replication prior to expression of late enzyme. However, no direct relation is observed between amounts of DNA and lysozyme synthesis in that some 7% of normal DNA synthesis occurs in T4rII-infected *E. coli* K(λ) (8), whereas only 1% of normal lysozyme synthesis occurs. Thus, even though early enzymes are made and DNA synthesis starts at the correct

time, the rII-infected cells can be shown to have abnormal metabolism prior to the cessation of DNA replication.

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LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Benzer, S. 1961. Genetic fine structure. Harvey Lect. 56.
3. Buller, C. S., and L. Astrachan. 1968. Replication of T4rII bacteriophage in *Escherichia coli* K-12(λ). J. Virol. 2: 298-307.
4. FerroLuzzi-Ames, G., and B. N. Ames. 1965. The multiplication of T4rII phage in *E. coli* K12(λ) in the presence of polyamines. Biochem. Biophys. Res. Commun. 18:639-647.
5. Garen, A. 1961. Physiological effect of rII mutations in bacteriophage T4. Virology 14:151-163.
6. Mark, K. K. 1970. A lysozyme assay method for low activity. Anal. Biochem. 37:447-450.
7. Mark, K. K. 1970. The relationship between the synthesis of DNA and the synthesis of phage lysozyme in *Escherichia coli* infected by bacteriophage T4. Virology 42:20-27.
8. Nomura, M. 1961. DNA synthesis in *Escherichia coli* K12(λ) after infection with an rII mutant of bacteriophage T4. Virology 14:164-166.
9. Rutberg, B., and L. Rutberg. 1966. Bacteriophage-induced functions in *Escherichia coli* K(λ) infected with rII mutants of bacteriophage T4. J. Bacteriol. 91:76-80.
10. Sekiguchi, M. 1966. Studies on the physiological defects in rII mutants of bacteriophage. J. Mol. Biol. 16:503-522.
11. Whitfield, J. F., and K. M. Baird. 1959. Nucleic acid synthesis by a lysogenic strain of *Escherichia coli* infected with an rII mutant of coliphage T2. Can. J. Microbiol. 5:17-24.