ON THE REGULATORY MECHANISM OF SYNTHESIS OF EARLY ENZYME INDUCED BY BACTERIOPHAGE T4[1/]

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One of the interesting problems for the biologist is the mechanism of the regulation of protein synthesis (see review by Jacob and Monod, 1961). The mode of synthesis of the so called early enzymes induced by T-even phage infection and participating in the production of viral DNA deserves attention in this respect. These enzymes are induced on introducing phage DNA (chromosome) into the host. Synthesis takes place only during the early stage of phage growth, coming to halt at about 15 minutes (Flaks et al., 1959; Kornberg et al., 1959). Presumably there is a "regulatory mechanism" which brings about cessation of enzyme synthesis.

The synthesis of enzyme induced by ultraviolet light (UV) irradiated phage does not cease at the normal time, but continues so that the final enzyme level exceeds that produced in the normal phage replication cycle (Dirksen et al., 1960; Delihas, 1961). It appears that the phage carries the information necessary to initiate enzyme synthesis along with something that regulates enzyme formation and is particularly susceptible to UV-irradiation.

The present work was undertaken to determine if the "regulatory mechanism" is mediated by cytoplasmic materials. If cytoplasmic materials are involved, the introduction of both UV-irradiated and non-irradiated phage

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must result in the dominance of the non-irradiated chromosome; that is the increase of the enzyme should level off at about 15 minutes after infection. The experiments were carried out by studying the formation of deoxycytidine triphosphatase (dCTPase) in Escherichia coli K12(λ).

MATERIALS AND METHODS

The wild type $T4r^+$ phage and two deletion type mutants of T4 (H88: deletion in A cistron, r196: deletion in B cistron; Benzer, 1955) were used. UV-irradiation was carried out by stirring the phage suspensions gently at a distance of 80 cm from a 15 watt Mazda germicidal lamp. A dose sufficient to produce 5 lethal hits was used. E. coli K12(λ) organisms grown to the late logarithmic phase with aeration at 37° C in a glucose-salts synthetic medium (Sekiguchi and Takagi, 1960) supplemented with 100μg/ml of DL-tryptophan were infected with phage at an input multiplicity of 10 phage particles per cell (zero time). Incubation was continued and aliquots of the infected culture were taken out at intervals, chilled to stop further synthetic reactions and centrifuged.

The collected cells were resuspended in 0.02 M Tris-acetate buffer, pH 7.5 and disrupted by sonication at 0° C for 2 minutes (20 KC sonic oscillator, Measuring and Scientific Equipment Ltd.). The sonicate was centrifuged at 12,000 xg for 10 minutes. Almost all the cell protein appeared in the supernatant which was used as the enzyme preparation.

Deoxycytidine triphosphate labeled with P^{32} in β and γ position was chemically synthesized according to the procedures described by Smith and Khorana (1958). The assay for dCTPase consisted of counting the charcoal-nonadsorbable P^{32} released after incubation with enzyme for 20 minutes (Zimmerman and Kornberg, 1961). Protein content was determined by a modified Folin reaction (Lowry et al. 1951). Enzyme activity was expressed as μmoles of inorganic pyrophosphate liberated from dCTP per minute per mg protein.

RESULTS AND DISCUSSION

In the present paper "limited synthesis" will refer to the synthesis of dCTPase that stops after 15 minutes of infection. Synthesis of enzyme

continuing beyond 15 minutes will be termed "unlimited synthesis".

Mixed infection with H88 and r196 as well as infection with r^+ alone resulted in "limited synthesis" of dCTPase. This is illustrated in Fig. 1 a and b solid lines.

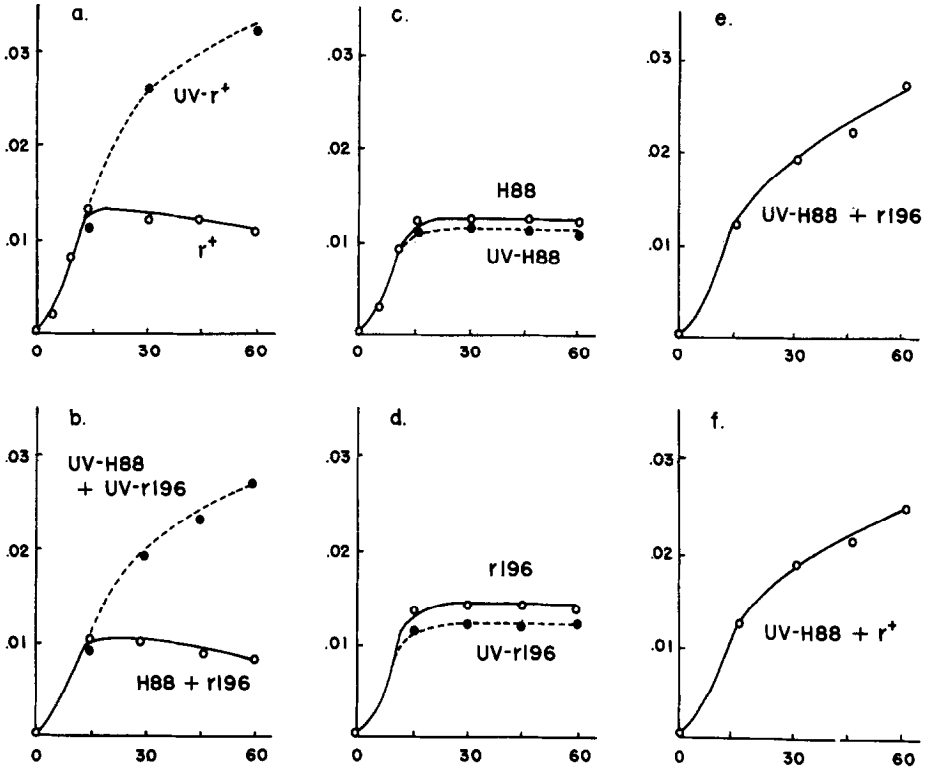

Figure 1. Kinetics of dCTPase synthesis in E. coli K12(λ) infected with bacteriophage T4.
The bacteria at 7 to 8 x 10^8/ml in synthetic medium were infected with various types of T4 phage as indicated in the figures at multiplicity = 10 in the case of a, c and d. In b, e and f the multiplicity of infection was 5 for each type of phage to make a total of 10.
Ordinate: dCTPase activity in μmoles PPi^{32} released / min / mg protein.
Absissa: Time in minutes after infection.

"Unlimited synthesis" was observed on infection with UV-irradiated r^+ or on mixed infection with UV-irradiated H88 and UV-irradiated r196 simultaneously (Fig. 1 a and b dotted lines). These observations confirm the data of Dirksen et al. (1960) who studied the mode of synthesis of the same enzyme in E. coli B infected with T2 phage.

The dotted lines of Fig. 1 c and d show that infection with either UV-irradiated H88 or with UV-irradiated r196 results in "limited synthesis". Therefore, there is some difference between the mechanism which stops enzyme formation in organisms infected separately with either of the rII bacteriophage mutants and that in organisms infected with wild type phage or mixedly with H88 and r196. Complementation, leading to restoration of the rII function, occurred in the mixed infection. The general loss of synthetic capacities in E. coli K12(λ) caused by the rII character (Garen, 1961; Nomura, 1961) may be responsible for the failure of "unlimited synthesis" to occur on infection with UV-irradiated rII mutants.

Infection of the host cell with any one of the following phages: r^+, H88, r196, UV-irradiated H88 and UV-irradiated r196 (but not UV-irradiated r^+), always leads to "limited synthesis". Very different results were obtained after simultaneous infection of bacteria with one UV-irradiated and another non-irradiated complementary rII mutant (UV-H88 + r196, or H88 + UV-r196). "Unlimited synthesis", that is extended and exaggerated production of the enzyme beyond 15 minutes was observed (Fig. 1 e) in such experiments. The same result was obtained when bacteria were infected with a UV-irradiated rII mutant and non-irradiated wild type phage (Fig. 1 f). It should be noted that under these experimental conditions, "unlimited synthesis" takes place only in those complexes mixedly infected with the two kinds of phages. Thus the UV-irradiated phage behaved as if it were "dominant" over the non-irradiated phage with respect to the mechanism for control of the level of infection induced dCTPase.

Wiberg et al. (1962) demonstrated with the am mutants of T4 phage that the occurrence of phage DNA synthesis is closely related to the cessation of the synthesis of the early enzymes. It seems that the accumulation of phage DNA is not the direct cause for the cessation of dCTPase synthesis, since "unlimited synthesis" of dCTPase took place in organisms mixedly infected with both UV-irradiated and non-irradiated phages, and such organisms support phage growth (and therefore DNA synthesis). Repression due to the accumula-

tion of enzymatic reaction products (feed back inhibition) or due to the accumulation of a "cytoplasmic repressor" (Jacob and Monod, 1961) may also be ruled out by the same data, since the UV-irradiated phage chromosome behaved as if it were dominant over the non-irradiated chromosome when both were present together. If such mechanisms were operating, it is likely that the non-irradiated chromosome behaves dominantly. Therefore some other hypothesis perhaps involving an operator (Jacob and Monod, 1961) on the phage chromosome or some functional differentiation of the phage DNA molecule in the course of phage growth will have to be sought to account for the regulation of early protein synthesis in phage infected bacteria.

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