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DNA Synthesis and Lambda Phage Development in a Lysogenic Strain of *Escherichia coli* K12*

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SUMMARY

The relationship between deoxyribonucleic acid (DNA) and protein synthesis during lambda (λ) phage development in ultraviolet (UV) induced *Escherichia coli* K12 was studied.

1) Induction of λ phage formation in the lysogenic strain of *E. coli* K12, thymineless mutant, did not eliminate the need of the bacteria for exogenous thymine. When thymine was added immediately after UV irradiation, DNA synthesis commenced after a lag period of 60 minutes, while bacteria starved of thymine for 60 or 90 minutes were able to synthesize DNA immediately after the addition of the nutrient. In the former condition, mature phage particles were released into the medium after 75 minutes incubation, but in the latter they were detected in the medium without a latent period. When protein synthesis in UV induced bacteria was inhibited by chloramphenicol at an early period of incubation, no phage DNA or particles were synthesized.

2) When a methionine requiring mutant of *E. coli* K12 had been starved of methionine, the 30 minutes lag period required for DNA synthesis was increased to a period corresponding to the period of methionine starvation, and progeny phages were liberated into the medium after a normal latent period reckoned from the time of methionine addition.

3) A UV induced lysogenic strain of *E. coli* W3101 (λ) was scarcely affected by mitomycin C at a concentration which was sufficient to inhibit DNA synthesis of a non-lysogenic strain of *E. coli* W3101.

INTRODUCTION

Infection of *Escherichia coli* with members of the T-even series of bacteriophages results in the cessation of bacterial DNA synthesis. After several minutes lag period, synthesis of phage DNA commences. When protein synthesis is blocked at an early stage during the latent period, no phage DNA is synthesized. However, when protein synthesis is suppressed a little later after infection, synthesis of phage DNA proceeds even if no further protein synthesis is taking place (Burton, 1955; Melechen, 1955; Tomizawa and Sunakawa, 1956). Recent studies on phage multiplication have demonstrated that number of new enzymes, which cannot

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be detected in the host cell, are formed during the first few minutes of infection with T-even phages (Cohen, 1961). Barner and Cohen have shown that infection of a thymine requiring mutant of *E. coli* with T2 phage results in a removal of the block in thymine synthesis (1954) and that the activity of thymidylate synthetase in a thymineless mutant of *E coli* is increased about 1000-fold by infection of the bacteria with T2 phage (1959).

However, we have still very little knowledge as to whether or not new proteins are formed before the synthesis of temperate phage DNA takes place (Miki and Matsushita, 1956; Thomas, 1959). This may be partly due to the difficulty of distinguishing between the DNA of a temperate phage and of its host, because phage DNA does not contain a specific compound such as 5-hydroxymethylcytosine which is peculiar to T-even phages. Since a thymine requiring mutant of *E. coli* K12 (Okada *et. al.*, 1960) and an antibiotic mitomycin C which inhitits bacterial DNA synthesis (Shiba *et. al.*, 1959) have become available, it is now possible to elucidate the relationship between the synthesis of DNA and protein during temperate phage multiplication. This paper describes the results obtained by analyse of DNA and protein synthesis in a UV induced lysogenic strain of *E. coli* K12 thymineless or methionineless mutant, together with those obtained by using antibiotics, chloramphenicol and mitomycin C.

MATERIALS AND METHODS

1. *Bacterial strains*

Strains of *E. coli* K12 (KT^-, thymineless*; 58-161, methionineless), both of which are lysogenic for λ prophage, were used in this experiment. *E. coli* W3101 (λ) was isolated after infecting a non-lysogenic strain of *E. coli* W3101 with the wild type strain of λ phage. As plating indicator for phage λ, *E. coli* W3101 was used.

2. *Media*

The bacteria were grown in a minimal medium (SG medium) containing 2.5 g of $(NH_4)_2HPO_4$, 1.5 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 5 g of NaCl, 2 g of sodium glutamate and 3 g of glucose per liter at pH 7.2. Thymine (10 μg/ml) and methionine (50 μg/ml) were added to the media for growth of the respective mutants. The indicator strain for phage titration was grown in nutrient broth medium (10 g meat extract, 10 g polypeptone, 1 g NaCl in 1 liter of H_2O and the pH was adjusted to 7.2 with NaOH).

3. *Phage assay*

E. coli W3101 at a concentration of about 10^9 per ml in nutrient broth medium was centrifuged and suspended in an AD solution (0.01 M phosphate buffer, pH 7.0, 0.01 M $MgSO_4$ and 0.085 M NaCl) at about the same concentration. The λ phage lysate was diluted to 10^2 with an AD solution and added to this bacterial suspension. After 30 minutes incubation at 37°C, the phage-bacterium complex was plated on *E. coli* W3101 by the soft agar layer method (Adams, 1950).

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* This strain was generously supplied by Dr. T. Okada of Kanazawa University.

4. *Chemical analysis*

RNA and DNA were assayed by the orcinol and diphenylamin methods, respectively (Mejbaum, 1939; Burton, 1956). Bacteria were chilled to 0°C and acidified by perchloric acid at final concentration of 0.5 N. The acid insoluble fraction was suspended in 0.5 N perchloric acid and heated at 90°C for 15 minutes. DNA and RNA in the extract were assayed and, after dissolving the residue in a small volume of 1 N NaOH, protein was determined by the method of Lowry *et al.* (1951).

5. *Chemicals*

Mitomycin C was provided from Kyowa Hakko Kogyo Company and chloramphenicol was purchased in a crystalline form from Sankyo Company.

6. *Irradiation with ultraviolet light*

UV irradiation of bacteria was carried out by exposure of the cell suspension to a 15 watt Toshiba germicidal lamp at a distance of 45 cm.

RESULTS

1. *DNA synthesis and λ phage development in the thymine requiring mutant*

E. coli KT⁻ cultures at a logarithmic phase of growth in SG medium with 10 μg per ml of thymine were harvested by centrifugation and suspended in SG medium.

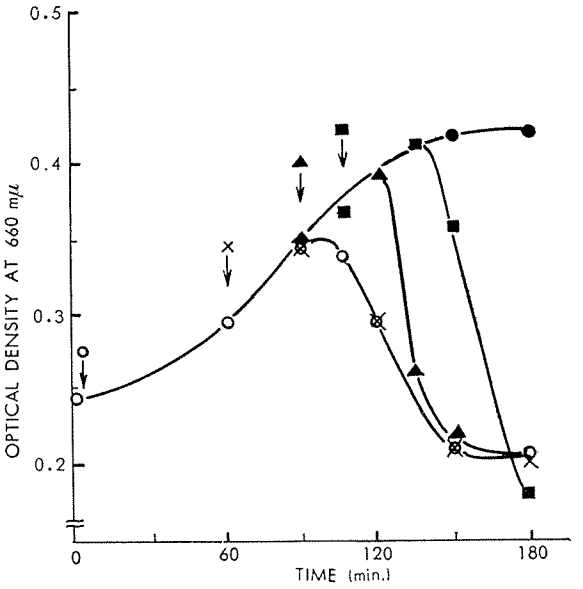

Fig. 1. Effect of Thymine Starvation on the Growth of *E. coli* KT⁻ after UV Irradiation

Logarithmically growing cells of *E. coli* KT⁻ were harvested and washed twice with saline. The cells were reincubated in SG medium in the absence of thymine and irradiated with UV for 45 seconds. The bacteria were shaken with vigorous aeration and thymine added (10 μg/ml) at the times indicated by the arrows.

○—○, at 0 minutes : ×—×, at 60 minutes : ▲—▲, at 90 minutes : ■—■, at 105 minutes : ●—●, no thymine.

After irradiation of the bacterial suspensions with UV for phage induction, they were shaken with aeration at 37° C, and thymine (10 μg/ml) was added at intervals. Fig. 1 shows the change in optical density of UV induced bacteria. It is apparent from the data that bacterial lysis begins at the same time in bacteria with thymine at the moment of UV irradiation and in bacteria after 60 minutes starvation of thymine. Under these conditions bacterial lysis began 90 minutes after UV irradiation. However, addition of thymine 90 or 105 minutes after UV irradiation resulted in residual growth for 30 minutes before bacterial lysis took place. In the absence of thymine, bacterial growth continued at the same rate as in the control culture, but no lysis occurred during the test period. The bacterial growth observed in Fig. 1 suggests that even in the absence of exogenous thymine, the bacteria are still able to synthesize bacterial components, but are unable to produce bacteriophage particles, because an increment in optical density was observed without lysis of the bacteria. This is clearly seen in Table 1 where, in the medium without thymine, there was no DNA synthesis or release of phages into the medium, but more RNA and protein were synthesized.

Table 1. Effect of Thymine Starvation on λ Phage Multiplication and Protein and Nucleic Acids Synthesis in *E. coli* KT$^-$

Exponentially growing *E. coli* KT$^-$ in SG medium at 37° C were harvested, washed twice and suspended in the same medium. The bacteria were irradiated with UV for 45 seconds and divided into two portions. One portion was incubated with thymine (10 μg/ml) and the other without thymine for 2 hours with vigorous shaking. Aliquots of the bacteria were removed for counting infective centers on λ phage sensitive bacteria and the rest was used for measuring the protein and nucleic acids.

Thymine (μg/ml)	Time of incubation (min.)	DNA-P (μg/ml)	RNA-P (μg/ml)	Protein (μg/ml)	Infective centers/ml
0	0	0.18	0.345	45.4	1.0×10^{8}*
0	120	0.20	0.760	75.8	5.0×10^{7}*
10	120	0.61	0.654	69.8	6.4×10^{9}

* corresponding to induced bacteria

These results show that RNA and protein were synthesized even in the absence of thymine and that lysis of bacteria began at the same time, if thymine was supplied after 60 minutes starvation. Therefore, the effect of thymine addition on the synthesis of cellular constituents and phage multiplication was studied.

E. coli KT$^-$ grown in SG medium supplemented with thymine were harvested and washed twice with saline. These cells were suspended in SG medium and irradiated with UV for phage formation. Then, they were divided into two portions, to one of which thymine (10 μg/ml) was added immediately after UV irradiation. The other portion was incubated without thymine for 60 minutes. Both were shaken with aeration at 37° C and aliquots were taken for the measurement

of nucleic acids and protein. It is clear from Fig. 2 that the amount of RNA and protein in thymine starved bacteria increased at the same rate as in the bacteria supplied with thymine throughout the experiment. If thymine was added immediately after UV irradiation, DNA synthesis did not occur for 60 minutes and thereafter proceeded linearly. However, addition of thymine after 60 minutes interval permitted an immediate synthesis of DNA (cf. Fig. 4). As is seen in Fig. 3, addition of thymine after 90 or 120 minutes starvation also results in the immediate synthesis of DNA. The latent period for λ phage multiplication is 75 minutes if thymine is supplied immediately after UV irradiation. However, if UV induced bacteria were starved of thymine with shaking and then transferred to a complete medium 60 to 150 minutes after UV irradiation, many mature phage particles were released into the medium during a further 30 minutes incubation period, showing that there is no latent period in the synthesis of λ phage and of DNA. At the present time it is impossible to discriminate between the chemical constituents of DNA of the temperate phage λ and of its host *E. coli* K12, but the result may indicate that, a portion if not all the DNA synthesized after UV

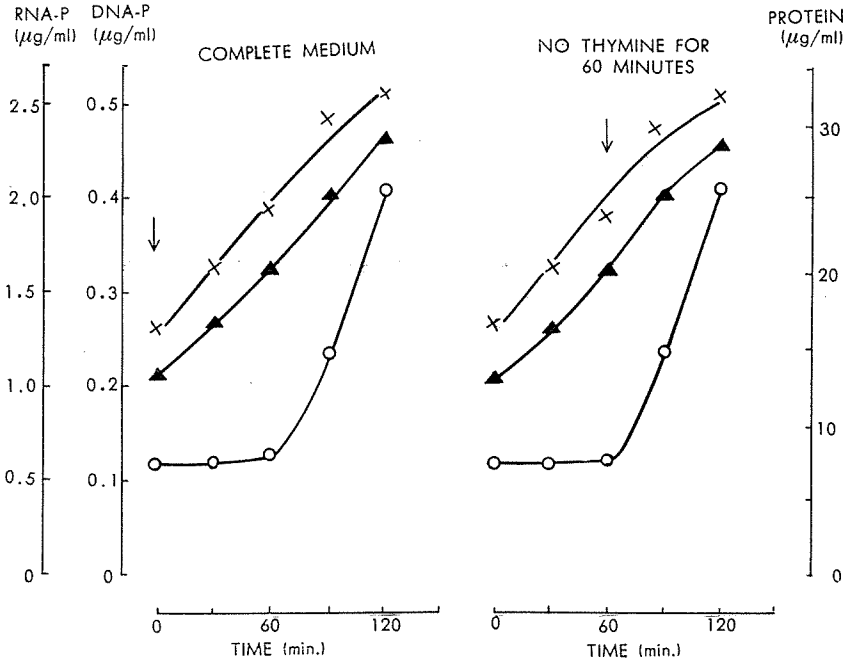

Fig. 2. The Synthesis of DNA, RNA and Protein in *E. coli* KT⁻ after Irradiation with UV

The experimental procedures were the same as those described in Fig. 1, except that thymine was added to one culture after 60 minutes starvation. Aliquots were taken at intervals for measurement of DNA, RNA and protein.

○—○, DNA : ×—×, RNA : ▲—▲, protein

induction of lysogenic bacteria is phage specific. These results also lead to the concept that a thymineless mutant of *E. coli* K12 can form various systems which are related to the synthesis of phage DNA and phage multiplication even in the absence of exogenous thymine.

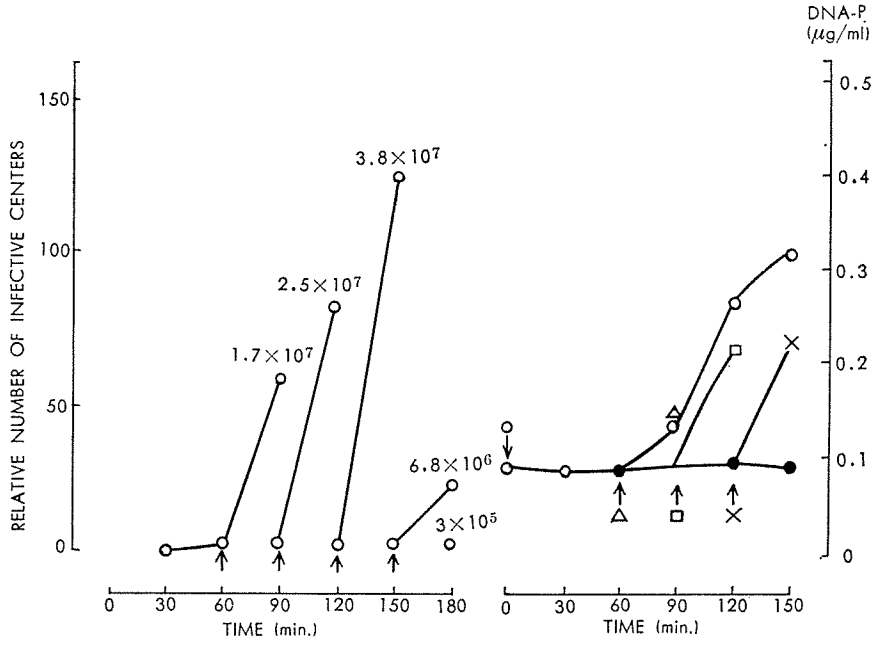

Fig. 3. Effect of Thymine Addition on λ Phage Development and DNA Synthesis in *E. coli* KT⁻ after a Given Time of Starvation

The conditions were the same as those used for Fig. 1. Thymine was added at the times indicated by the arrows and aliquots of the samples were removed after 30 minutes incubation for assay of the phage titer and the amount of DNA. The relative number of infective centers means the ratio of infective centers after 30 minutes incubation to those at the time of thymine addition.

In order to clarify the above system, chloramphenicol was used, in view of the idea that the system may consist of protein molecules which participate in phage DNA synthesis. The principle of the experiment was as follows; If the synthesis of phage DNA does not require prior protein synthesis, DNA and phage formation by UV induced *E. coli* KT⁻, which have been exposed to chloramphenicol in thymine starved SG medium at an early period during incubation, after which the drug is removed by transferring the cells to complete midium, will commence immediately after the transfer. However, if it requires prior protein synthesis, after the same treatment, there will be a noraml latent period before DNA and phage synthesis. The results of the experiment performed along this line are shown in Table 2. When chloramphenicol was added early during the incubation period and then the bacteria

were transferred after 90 minutes to SG medium containing thymine, a little phage and DNA was synthesized during a further 30 minutes incubation period. But, when UV induced bacteria were exposed to the drug 60 minutes after induction, at a time when a little protein had already been synthesized, there was a relative big increases in DNA and phage formation. It is concluded that there is a chloramphenicol sensitive stage in phage DNA synthesis.

Table 2. Effect of Chloramphenicol on λ Phage Multiplication in UV Induced *E. coli* KT⁻

Washed bacteria were irradiated with UV for 45 seconds and shaken with vigorous aeration in SG medium lacking thymine. Chloramphenicol (20 μg/ml) was added at intervals and the cells were removed after 90 minutes by centrifugation. Then the bacteria were incubated in a plete medium with shaking for 30 minutes.

Time of Addition (min.)		Phages/ml at		DNA-P/ml at	
CM*	Thymine	90′	120′	90′	120′
0	90	1.4×10^5	4.0×10^5	0.129	0.110
30	90	3.0×10^5	3.4×10^6	0.129	0.145
60	90	3.5×10^5	1.1×10^7	0.159	0.255
75	90	4.0×10^5	3.2×10^7	0.191	0.343
none	90	8.0×10^5	1.5×10^8	0.205	0.408

CM : chloramphenicol

2. *DNA synthesis and λ phage development in a methionine requiring mutant*

To confirm the participation of protein synthesis in the initiation of phage DNA synthesis, a methionine requiring mutant of *E. coli* K12 was used. If protein synthesis participates in phage DNA synthesis as deduced from the above experiment, DNA and phage formation in a methionineless mutant will be delayed for a period corresponding to the period of methionine starvation, because they are unable to synthesize phage specific proteins in the absence of external methionine.

The experimental procedures were in principle the same as those for Fig. 2 except that methionine (50 μg/ml) was added in place of thymine. It is clear from Fig. 4 that protein synthesis occurred immediately after the addition of methionine, while before the synthesis of DNA there was a lag of about 30 minutes, even if methionine was present throughout the experiment or added 30 minutes after UV irradiation. The bacteria which were incubated with methionine after 30 minutes starvation had a lag before phage growth corresponding to the period of methionine starvation, releasing progeny phages after a normal latent period reckoned from the time of methionine addition. This result also appears to favor the the view that prior protein synthesis is necessary for the initiation of phage and its DNA formation.

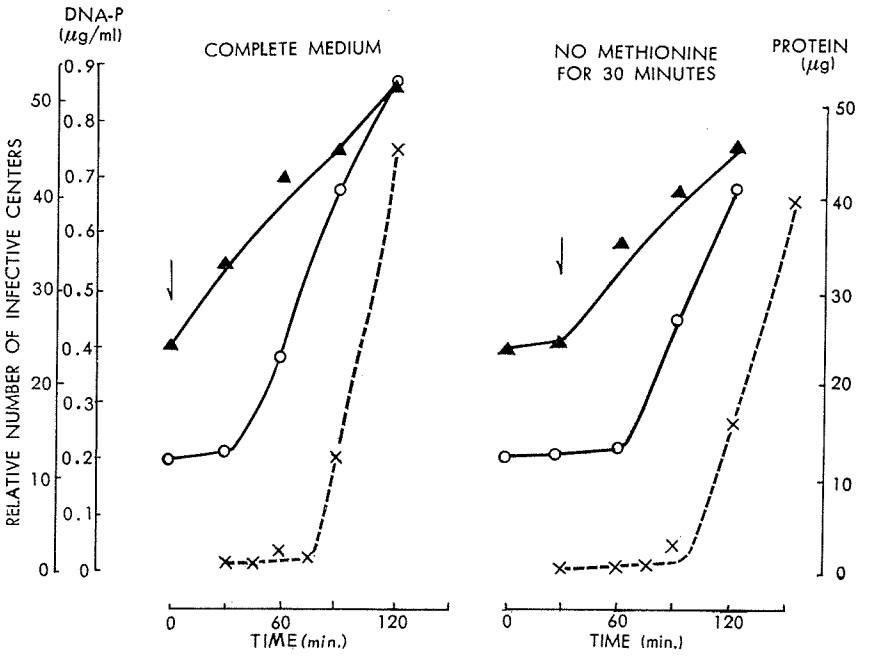

Fig. 4. Effect of Methionine Addition on λ Phage Development and DNA and Protein Synthesis in a Methionine Requiring Mutant of *E. coli* K12

The experimental procedures were the same as those used in Fig. 1 except that methionine (50 μg/ml) was added at the time indicated by arrow.
○—○, DNA : ▲—▲, protein : ×···×, relative number of infective centers

31 *DNA synthesis and λ phage development in the presence of mitomycin C*

The antibiotic mitomycin C has been reported to have a selective inhibitory action on bacterial DNA synthesis without exerting any effect on RNA and protein

Table 3. Effect of Mitomycin C on DNA Synthesis and λ Phage Development of *E. coli* W3101

E. coli W3101 (10 ml of 3.0×10^8 cells/ml) and UV induced *E. coli* W3101 (λ) (10 ml of of 5.2×10^8 cells/ml) were exposed to various concentrations of mitomycin C in SG medium for 2 hours.

Conc. of MC*	Time of incubation (min.)	DNA-P (μg/ml) W3101	DNA-P (μg/ml) W3101 (λ)	Phages/ml W3101(λ)
0	0	0.110	0.201	– – –
0	120	0.298	0.420	1.7×10^9
0.25	120	0.094	0.410	1.9×10^9
0.5	120	0.069	0.390	1.4×10^9
1.0	120	0.048	0.375	1.4×10^9
2.0	120	0.042	0.360	1.2×10^9

* MC : mitomycin C

synthesis (Shiba *et al.*, 1959). Infection of *E. coli* B with the T series of bacteriophages resulted in restoration of the blockage in DNA synthesis produced by mitomycin C (Sekiguchi and Takagi, 1959). Table 3 shows the effect of mitomycin C on DNA synthesis in UV induced lysogenic and non-lysogenic strains of *E. coli* W3101. *E. coli* W3101 and W3101(λ) at an exponential growth phase were harvested by centrifugation. After washing, *E. coli* W3101(λ) was exposed to UV and incubated in SG medium with various concentrations of mitomycin C for 120 minutes. A concentration of 0.25 μg per ml of mitomycin C resulted in the complete inhibition of DNA synthesis in a non-lygosenic strain, while the presence of higher levels of the drug did not affect the synthesis of DNA and λ phage in a UV induced lysogenic strain.

DISCUSSION

Several experiments have suggested the dependence of DNA synthesis on prior protein synthesis. Harold and Ziporin in their investigations on nucleic acid and protein synthesis in UV or mustard treated *E. coli*, demonstrated that protein synthesis was required for the initiation of DNA synthesis (1958). In irradiated bacteria, the presence of chloramphenicol or of amino acid analogues leads to a decrease in the yield of the mutant, indicating that protein synthesis must occur before the DNA is duplicated in order to produce mutation (Witkin, 1956; Doudney and Haas, 1959; Schwartz and Strauss, 1958,). Dependence of DNA synthesis upon prior protein synthesis was analysed in detail in studies on the multiplication of the T-series of bacteriophages. DNA synthesis in bacteria infected with Teven phages is completely inhibited if inhibitors of protein synthesis are added before or immediately after infection (Burton, 1955; Melechen, 1955; Tomizawa and Sunakawa, 1956). If, however, the bacteria are infected with phages and then exposed to inhibitors of protein synthesis only a few minutes after the start of intracellular phage development, DNA synthesis proceeds without further protein synthesis. The data obtained in the present series of experiments, using chloramphenicol and methionine or thymine requiring mutants, also appear to favor the view that during the multiplication of temperate phage λ, protein synthesis is necessary for the initiation of DNA and phage formation.

We must now consider the question of whether the need for protein synthesis is specifically related to phage DNA synthesis. As seen in Fig. 3, DNA synthesis is immediately followed by the concomitant synthesis of λ phage particles when thymine is supplied to UV induced *E. coli* KT^- after several minutes of incubation. This suggests that some if not all the DNAs synthesized during temperate phage multiplication is λ phage DNA, although we cannot distinguish between the DNA of λ phage and of *E. coli* K12. When *E. coli* KT^- was incubated in a thymine deficient SG medium with chloramphenicol immediately after UV irradiation, and then 90 minutes later resuspended in complete medium without the drug,

there was no synthesis of DNA and λ phage during a further 30 minutes period (Table 2). From these results it may conclude that at least a portion of the proteins synthesized during the first few minutes after UV induction may be specifically related to the synthesis of λ phage DNA.

The question now is, what is the mechanism which confers on the UV induced lysogenic strain the ability to overcome the inhibition of DNA synthesis produced by mitomycin C. If we accept the view presented above and assume that induction of λ phage development in lysogenic *E. coli* induces the synthesis of new enzymes catalyzing an alternate pathway for DNA synthesis which is no longer affected by mitomycin C as in T2 bacteriophage (Sekiguchi and Takagi, 1960; Aposhian and Kornberg, 1961), or induces sufficient enzymes to overcome the inhibition caused by mitomycin C, it is possible to explain the peculiar phenomena obtained in the analysis of the effect of mitomycin C on non-lysogenic and UV induced lysogenic strains of *E. coli*.

Restoration of impaired synthesis of DNA with UV induction of lysogenic bacteria is also explainable on the assumption proposed by Reich *et al.* (1961) that the primary action of the drug may be to damage the DNA molecule and that damaged DNA can not act as a DNA template. However, if we accept this view, we must assume that bacterial DNA is more susceptible to the action of mitomycin C than λ phage DNA, in spite of the fact that the chemical constituents of λ phage DNA are similar to those of the host bacterium.

It was reported that infection of thymine requiring mutant of *E. coli* with T even or T5 phages is able to eliminate the need for exogenous thymine of the bacteria and confer on them the ability to synthesize phage DNA (Barner and Cohen, 1954 ; Crawford, 1959). However, it is apparent from Table 1 that induction of λ phage development in a lysogenic strain of a thymineless mutant cannot eliminate the thymine requirement of the bacteria. Infection of a virulent mutant of λ phage is also unable to endow the bacteria with the ability to produce bacteriophage particles if the exogenous requirements of the bacteria were omitted from incubation medium. These results may be explained on the hypothesis that the DNA of T-even and T5 phages contain a polynucleotide sequence which controls the synthesis of thymidylate synthetase (Barner and Cohen, 1959), but λ phage may lack this sequence. Therefore, we may suppose that, even though phage specific proteins are synthesized after UV induction of lysogenic bacteria, the enzyme systems catalyzing thymine synthesis are not produced, and hence that the thymine moiety in the phage DNA is formed through a reaction catalyzed by the enzyme systems of the host cells.

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