GENETIC AND PHYSIOLOGICAL STUDIES OF BACTERIOPHAGE T5
2. THE RELATIONSHIP BETWEEN PHAGE DNA SYNTHESIS AND PROTEIN SYNTHESIS IN T5-INFECTED CELLS

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SUMMARY

When Escherichia coli is infected with a mutant of T5 that does not synthesize detectable amounts of phage DNA, the three groups of protein that can be detected in a wild-type infection, namely, pre-early, early, and late, are all synthesized. Furthermore, the times of onset and cessation of their syntheses are essentially the same as in wild-type infections. It appears, therefore, that T5, unlike T-even, does not require replication of its DNA in order to induce synthesis of its late proteins.

INTRODUCTION

In bacteria infected with wild-type phage T2 or T4, the cessation of synthesis of early proteins and the onset of synthesis of late proteins depends upon phage DNA synthesis (1, 2). Furthermore, if phage DNA synthesis ceases, the synthesis of late proteins also ceases shortly thereafter (3-5). Thus, in T-even infected cells, continued synthesis of late proteins seems to require sustained synthesis of phage DNA. This requirement for DNA synthesis, however, can be circumvented if cells are infected with a double mutant of T4 which is defective in the genes controlling both phage-induced DNA-ligase and deoxyribonucleases (DNases) (6, 7). Because T5 exhibits certain unique features that are not shared by more extensively studied phages, it was of interest to determine if T5 also requires phage DNA synthesis for the normal kinetics of early and late protein synthesis.

MATERIALS AND METHODS

Bacteria and bacteriophages: Escherichia coli F is a fast adsorbing strain for coliphage T5, but is a non-permissive (su^-) host for amber phage mutants (8). The isolation of D9·amH18a, the amber mutant of T5 that is used in this study, is described in reference 9.

Media: Bacteria were grown in M-9 medium supplemented with 10 µg per ml of each of 18 amino acids (Arg, Lys, His, Try, Tyr, Phe, Pro, Gly, Ala, Val, Leu, Ileu, Ser, Thr, Cys, Met, Asp, Glu). M-9 medium that lacks utilizable sources of carbon and nitrogen was used as adsorption buffer. All media used for adsorption and growth of T5 contained 6×10^{-4} M $CaCl_2$ and 0.01 % gelatin.

Determination of rates of DNA synthesis in T5-infected cells: Cells of E. coli F were grown to 2×10^8 per ml in supplemented M9 medium, centrifuged, and resuspended in adsorption buffer at 5×10^9 per ml. After a 45 min period of incubation at 37° with aeration to exhaust residual nutrients, the bacteria were infected with phage at an input ratio of about 5 plaque-forming-units per cell. After a 10 min period of adsorption, the suspension was diluted 10-fold into supplemented M9 medium, and aeration was begun. One-ml samples were removed at specified times and rapidly mixed with 0.1 ml of supplemented M-9 medium containing 0.2 µCi of 2-^{14}C-thymidine (at a specific activity of 50 Ci per mole), 200 µg deoxyadenosine, and 4 µg uracil in a separate tube that had been equilibrated at 37°. After exactly 2 min of incubation at 37°, 1 ml of cold 10% CCl_3COOH was added and the tubes were placed in ice. The precipitates were filtered through Millipore filters and were washed extensively with cold 5% CCl_3COOH. After drying, the filter discs were assayed for radioactivity in a liquid scintillation counter.

Determination of patterns of protein Synthesis: Cells were grown and infected as for the determination of rates of DNA synthesis. Ten-ml samples were removed at specified times and rapidly mixed with 0.2 ml of a solution containing 2.0 µCi of ^{14}C-L-leucine (at a specific activity of about 250 Ci per mole) in a separate tube that had been equilibrated at 37°. After exactly 2 min of incuba-

tion at 37°, the contents of each tube were poured over cracked ice. The samples were centrifuged and the pellet was resuspended in 2 ml of 0.01 M Tris buffer at pH 7.4 containing 0.01 M $MgCl_2$. The soluble proteins were liberated by disrupting the cells in a Branson sonifier. Disc-gel electrophoresis and autoradiography were carried out as described by McCorquodale and Buchanan (10).

RESULTS

D9·amH18a is an amber mutant of T5 that is defective in phage-induced DNA polymerase (9). The essential role that this enzyme plays in normal T5 DNA synthesis is illustrated by the finding that this mutant does not synthesize detectable amounts of DNA in a non-permissive cell (Fig. 1). The very small increase in rate seen after 80 min probably represents DNA synthesis in the few cells that escaped phage infection and continued to grow. The rate of synthesis of T5 DNA in $T5^+$-infected cells is shown for comparison.

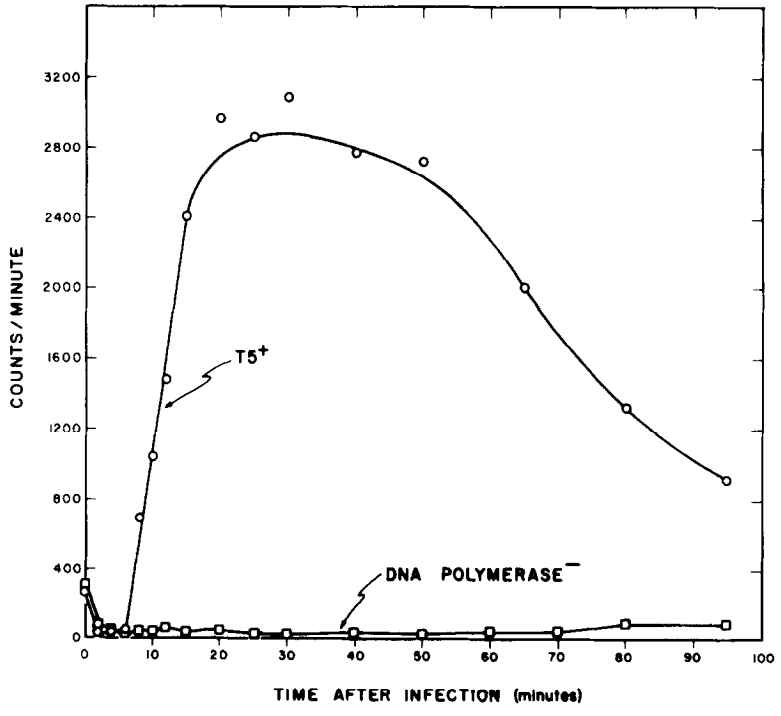
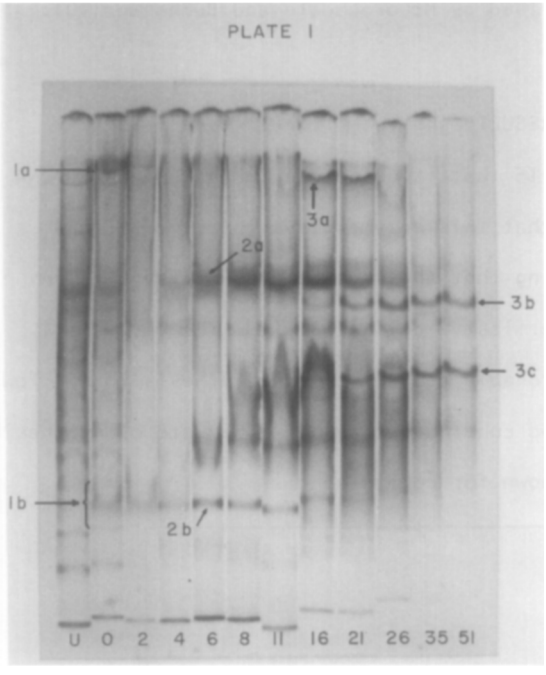
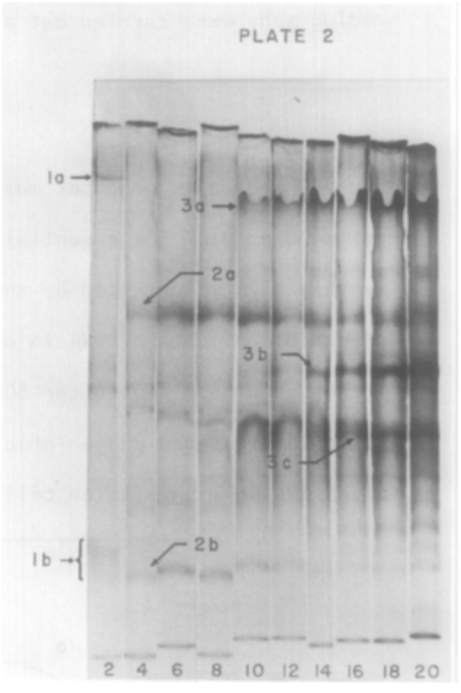


Fig. 1. Kinetics of DNA synthesis in E. coli F cells infected with $T5^+$ (open circles) and with D9·amH18a (DNA polymerase$^-$, open squares). The amount of ^{14}C-thymidine converted to an acid-precipitable form during 2 min periods of incubation is shown as a function of time after infection.





Plates 1 and 2. Autoradiograms of electrophoretically separated ^{14}C-L-leucine-labeled proteins synthesized in E. coli F infected by T5^{+} (Plate 1) and by D9·amH18a (Plate 2). "U" represents uninfected cells labeled for 2 min, and the number below each gel indicates the time after infection that the 2-min labeling period was begun.

The effect of the lack of T5 DNA replication on the synthesis of the three classes of T5 proteins previously reported (10) is shown in Plates 1 and 2. The pattern of protein synthesis induced by T5^{+} (Plate 1) represents the control, in which T5 DNA replication proceeds normally, and consists of three groups of proteins that are characterized by their times of synthesis (10). Synthesis of pre-early proteins begins during the first 2 min after infection and ceases by

6 to 8 min. This group is represented by bands 1a and 1b. Synthesis of early proteins, represented by bands 2a and 2b, begins between 4 and 6 min after infection and is substantially arrested by about 20 min. Synthesis of late proteins, represented by bands 3a, 3b, and 3c, begins between 10 and 12 min after infection and continues until lysis. This wild-type pattern of T5 protein synthesis is also found in D9·amH18a-infected cells (Plate 2), in which no detectable DNA synthesis takes place. The most striking result is, therefore, that late proteins are synthesized when no phage DNA synthesis occurs. It should also be noted that the shut-off of early protein synthesis (bands 2a and 2b in Plate 2) appears normal. Since the intensities of corresponding bands in Plates 1 and 2 are very similar, the differences in the amounts of protein synthesized in $T5^+$- and in mutant-infected cells is probably rather small.

DISCUSSION

Zweig et al. (11) reported that no empty T5 heads are produced in cells infected with $T5^+$ in the presence of 0.2 M hydroxyurea. Since phage DNA synthesis was not observed in these cells, it was suggested that the synthesis of T5 structural (i.e., late) proteins is linked to the synthesis of phage DNA. In view of our present results, we think the experiments of Zweig et al. probably indicate that the inhibition of protein synthesis and of DNA synthesis by high concentrations of hydroxyurea occur by independent mechanisms. The recent demonstration that empty T5 heads are produced in non-permissive cells infected with D9·amH18a (Zweig, personal communication) confirms the findings reported in this paper. Also, Dr. J. M. Buchanan (personal communication) has informed us that near-normal levels of T5-induced lysozyme (a late protein) accumulate in non-permissive cells infected with a polymeraseless mutant of T5. Thus, it seems quite clear that in T5-infected cells, the normal regulation of protein synthesis is not dependent upon phage DNA synthesis.

In the case of T4, the requirement of DNA synthesis for late protein synthesis can be circumvented by using multiple mutants that induce both defective

DNA-ligase and defective nucleases (6, 7). Mutants of this type probably allow single-strand nicks to exist in parental DNA *in vivo* without degradation of the phage DNA. Since parental T5 DNA already contains single-strand nicks (12, 13), it may be that their continued presence *in vivo* permits the synthesis of late T5 proteins in the absence of phage DNA synthesis. Certain features of the genetic map of T5 suggest that the nicks may be present during a substantial portion of the latent period (9, 14). Investigation into the fate and function of the single-strand nicks in T5 DNA is currently in progress.

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