THE ROLE OF PROGENY VIRAL DNA IN THE REGULATION OF ENZYME AND DNA SYNTHESIS*

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Infection of E. coli with T-even bacteriophages (Cohen, 1961), as well as infection of mammalian cells with herpes simplex virus (Keir and Gold,1963), vaccinia virus (McAuslan and Joklik, 1962), and pseudorabies virus (Nohara and Kaplan, 1963), leads to an increase in the activity of some of the enzymes concerned with the synthesis of DNA. The rise in the level of enzyme activity occurs during the early stages of the infectious process only, and then ceases, unless a mechanism which normally regulates the level of these enzymes is disturbed. This situation is obtained when the infecting virus is UV-irradiated (Dirksen et al., 1960; Delihas, 1961; McAuslan, 1963), when viral DNA synthesis is inhibited by amethopterin (McAuslan, 1963) and when bacteria are infected with certain T4 amber mutants which do not allow the synthesis of viral DNA to proceed at a normal rate (Wiberg et al., 1962). Thus, the control of the synthesis of the early enzymes appears to be correlated with the level of progeny viral DNA.

The experiments to be described were designed to determine whether the controlling factor in enzyme synthesis is the level of viral DNA itself, or whether viral DNA competent to give certain information has

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to accumulate in the infected cell. Using the pseudorabies (Pr) virus-rabbit kidney (RK) cell system (Kaplan, 1957), we attempted to answer this question by substituting 5-bromodeoxyuridine (BUDR) for thymidine in the viral progeny DNA.

Monolayer cultures of RK cells in the stationary phase of growth do not synthesize DNA; when these cells are infected with Pr virus they synthesize only viral DNA (Ben-Porat and Kaplan, 1963), so that BUDR supplied at the time of infection is incorporated only into viral DNA. Stationary phase cells were infected with a high multiplicity of Pr virus. They were then incubated in Eagle's medium (+ 3% dialyzed serum) containing 5-fluorouracil (FU) (10 µg/ml to inhibit the synthesis of thymidylic acid) and 100 µg of BUDR per ml. Under these conditions, BUDR substitutes for 80% of the thymidine normally present in the progeny viral DNA, as determined by the method of Baldwin and Shooter (1963). As a control, cultures were treated identically but were incubated with 100 µg of thymidine per ml instead of BUDR. (The results obtained with these control cultures were indistinguishable from those obtained with infected cells to which neither FU nor thymidine was added.)

Table 1

Effect of Incubation with BUDR on Viral DNA Synthesis and on Virus Synthesis

Treatment	Infectivity (PFU/ml*)	Adenine-8-C^{14} in DNA (cpm/sample)	DNAase resistant adenine-8-C^{14} (cpm/sample)
BUDR	6.2×10^6	2115	595
Thymidine	7×10^8	1060	660

* PFU = plaque forming units
Cells were infected, incubated with adenine-8-C^{14} (0.2 µc/plate), and with either thymidine or BUDR. 16 hours after infection, the number of PFU produced by the cultures, as well as the amount of adenine-8-C^{14} incorporated into DNA in a DNAase sensitive or resistant form, was determined as described previously (Kaplan and Ben-Porat, 1961).

Table 1 shows that the number of infectious virus particles produced by the infected cells which were supplied with BUDR is greatly reduced; that DNA synthesis under these conditions can occur, as judged by the incorporation of adenine-8-C^{14} into DNA; and that part of the DNA becomes integrated into an organized form, since it becomes resistant to the action of deoxyribonuclease (DNAase).

BUDR-treated infected cells accumulate viral DNA, yet do not produce infectious viral particles, so that this DNA is probably incompetent with respect to the transfer of certain information. Thus, conditions are created which allow us to answer the question posed above.

Fig. 1 shows the increase in the level of enzymatic activity during the early stages of the infectious cycle in BUDR-treated and untreated

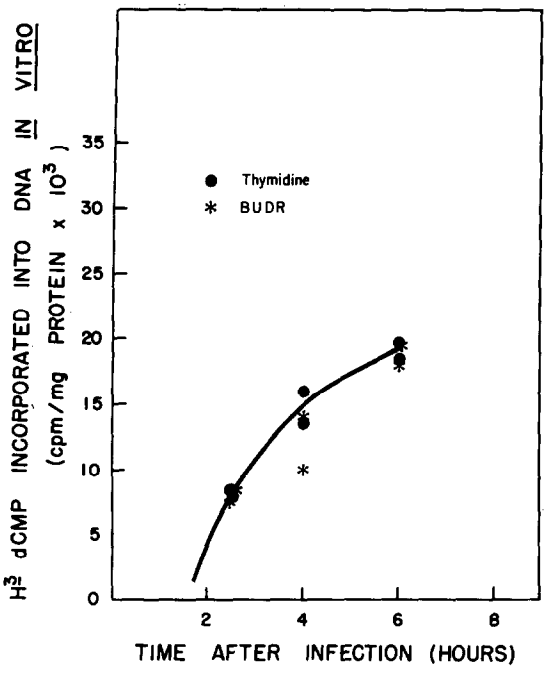

Fig. 1 Ability of extracts of infected cells incubated with BUDR or thymidine to incorporate H^3-dCMP during the early stages of the infective process. The preparation of the enzyme extracts and the assay procedure were described previously (Nohara and Kaplan, 1963).

control cultures. The incorporation of BUDR into the progeny viral DNA has no effect on the initial increase in enzymatic activity which follows infection. However, whereas no further increase occurs normally beyond 6 hours after infection and the level of enzyme activity remains constant for the next 4 hours (Fig. 2), the substitution of

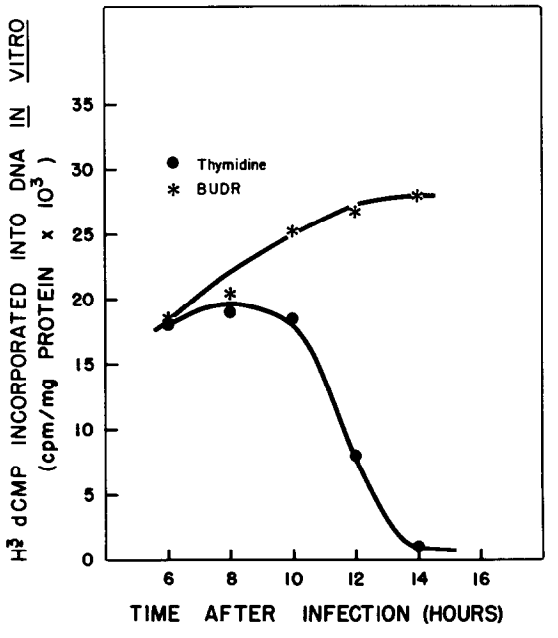

Fig. 2 Ability of extracts of infected cells incubated with BUDR or thymidine to incorporate H^3-dCMP during the late stages of the infective process.

BUDR for thymidine in the progeny DNA allows the increase in enzyme activity in the infected cells to continue up to 14 hours after infection, when normally there has been a loss of enzyme activity. The results in Fig. 1 and 2 illustrate the ability of the enzyme preparations to incorporate H^3-deoxycytidine monophosphate into DNA. Similar results are obtained when the activity of thymidine kinase or thymidine monophosphate kinase is determined.

The effect of the incorporation of BUDR into progeny viral DNA on

the ability of cells to synthesize DNA *in vitro* is closely paralleled by its effect on DNA synthesis *in vivo*. Whereas, normally, DNA synthesis stops approximately 12 hours after infection, as determined by the incorporation of adenine-8-C^{14} into DNA, BUDR-treated cells synthesize DNA linearly up to 16 hours after infection (Fig. 3). Similar results are obtained when the incorporation of P^{32} into DNA is followed.

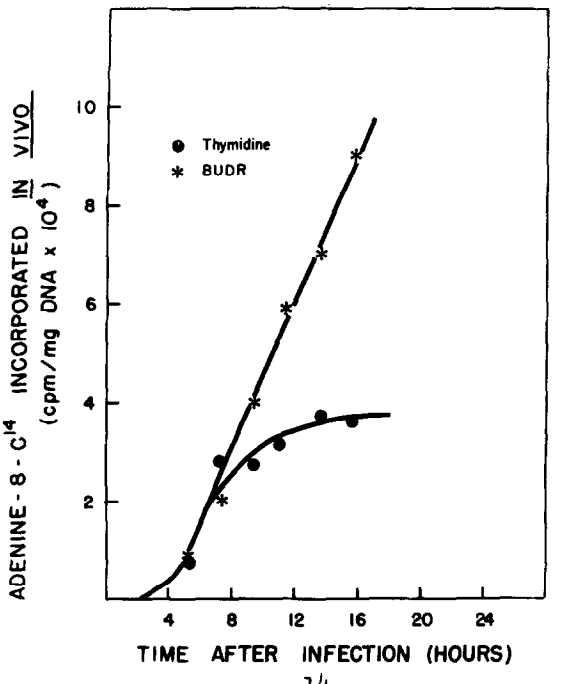

Fig. 3 Incorporation of adenine-8-C^{14} into the DNA of infected cells treated with BUDR or with thymidine. Infected cultures were incubated in Eagle's medium + 3% dialyzed serum, FU (10 µg/ml), and adenine-8-C^{14} (0.2 µc/culture). To one set of cultures thymidine (100 µg/ml) was added; to the other BUDR (100 µg/ml). At various times after infection, cultures were harvested and the amount of adenine-8-C^{14} incorporated into the DNA was determined as described previously (Kaplan and Ben-Porat, 1961).

Thus, substitution of BUDR for thymidine in viral progeny DNA interferes with the mechanisms regulating the level of enzyme activity and the rate of viral DNA synthesis in the infected cells.

Our results show, therefore, that the mechanism controlling these parameters is not the level of DNA per se but is dependent upon the presence of competent DNA.

References

Baldwin, R. L. and Shooter, E. M., J. Mol. Biol. 7, 511 (1963).
Ben-Porat, T. and Kaplan, A. S., Virology 20, 310 (1963).
Cohen, S. S., Fed. Proc. 20, 641 (1961).
Delihas, N., Virology 13, 242 (1961).
Dirksen, M. L., Wiberg, J. S., Koerner, J. F. and Buchanan, J. M., Proc. Nat. Acad. Sci. U. S. 46, 1425 (1960).
Kaplan, A. S., Virology 4, 435 (1957).
Kaplan, A. S. and Ben-Porat, T., Virology 13, 78 (1961).
Keir, H. M. and Gold,E., Biochim. et Biophys. Acta 72, 263 (1963).
McAuslan, B. R., Virology 20, 162 (1963).
McAuslan, B. R. and Joklik, W. K., Biochem. and Biophys. Research Commun. 8, 486 (1962).
Nohara, H. and Kaplan, A. S., Biochem. and Biophys. Research Commun. 12, 189 (1963).
Wiberg, J. S., Dirksen, M. L., Epstein, R. H., Luria, S. E. and Buchanan, J. M., Proc. Nat. Acad. Sci. U. S. 48, 293 (1962).