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Bacillus subtilis DNA Polymerase III Is Required for the Replication of the Virulent Bacteriophage ϕe

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The virulent phage ϕe of *Bacillus subtilis* which contains hydroxymethyluracil in its DNA requires host DNA polymerase III for its DNA replication. DNA polymerase III^{ts} mutant cells infected with ϕe at restrictive temperatures do not support phage DNA synthesis. However, ϕe grows normally both at low and high temperatures in the mutant's parent strain and in spontaneous DNA polymerase III^{+} revertants isolated from the mutant strain. Temperature-shift-down experiments with ϕe-infected cells having thermosensitive DNA polymerase III (*pol* III^{ts}) indicate that at 48 C the thermolabile DNA polymerase III is irreversibly inactivated and has to be synthesized de novo after the shift to 37 C, before phage DNA synthesis can begin. Temperature-shift-up experiments with ϕe-infected mutant cells show that phage replication is arrested immediately after the temperature shift and indicate that ϕe requires DNA polymerase III throughout its replication stage.

Bacteriophage ϕe contains hydroxymethyluracil in its DNA in place of thymine. This phage belongs to a group of large, double-stranded DNA phages of *Bacillus subtilis* (1, 11, 14, 21, 26). The lytic cycle of ϕe has been characterized (13, 24-26). Roscoe (25) found that host DNA synthesis is arrested in ϕe-infected cells several minutes after infection and phage DNA synthesis begins. In both ϕe and phage SPO1, which is related to ϕe, several observations indicate that phage DNA synthesis depends on the synthesis of a phage-specific DNA polymerase. Thus it was found, that a new DNA polymerase appears in SPO1-infected cells (32, 33). Both ϕe and SPO1 have been found to grow normally in DNA polymerase I^{-} mutant cells, indicating that DNA replication of these two phages does not depend on the host DNA polymerase I (16, 32). Moreover, DNA synthesis of ϕe and SPO1 is resistant to the drug 6-(*p*-hydroxyphenylazo)-uracil (HPUra) (18, 27), which inhibits DNA polymerase III (2, 10, 20). This suggests that DNA synthesis of these two phages does not require DNA polymerase III. Similar results were obtained with other *B. subtilis* phages like SPO2 (27) and PBS2 (23).

We show in this paper that host DNA polymerase III is required for ϕe replication throughout the stage of phage replication.

We will discuss the apparent contradiction between our results and other studies based on HPUra inhibition of DNA polymerase III (16, 18, 27), which indicate that this enzyme is not involved in ϕe DNA synthesis.

MATERIALS AND METHODS

Materials and methods, unless otherwise indicated, were as previously described (18).

Bacterial strains. The following strains of *B. subtilis* 168 were used: 2344 (*polA*$^{-}$, *thy*$^{-}$) designated 2344*pol III*$^{+}$ (2) and 2355 (*polA*$^{-}$, *polC*^{ts}, *met*$^{-}$) designated 2355*pol III*^{ts} (3) were described by Bazill and Gross. Strains U2355 (*polC*^{ts}, *met*$^{-}$, *ile*$^{-}$) designated U2355*pol III*^{ts} and R2355 (*polA*$^{-}$, *met*$^{-}$, *ile*$^{-}$) designated R2355*pol III*$^{+}$ were derived from strain 2355.

Phage strains. Phage ϕe wild-type strain and phage SPO1 wild-type strain were used.

Media. The standard growth medium used throughout the experiments was C^{+} (22).

One-step growth experiments. These experiments were performed as described by Sonenshein and Roscoe (29).

RESULTS

Kinetics of phage DNA synthesis in a DNA polymerase III^{ts} mutant strain. Preliminary experiments have shown that ϕe does not direct its DNA synthesis in either ϕe-infected U2355 *pol III*^{ts} (DNA polymerase III^{ts}) cells or in ϕe-infected 2355 *pol III*^{ts} (DNA polymerase III^{ts}, DNA polymerase I^{-}) cells at 48 C. On the other hand, phage DNA synthesis is normal at 48 C in ϕe-infected 2344 *pol III*$^{+}$ (DNA polymerase I^{-}) cells. We therefore performed our experiments with the double mutant strain 2355*pol III*^{ts} (DNA polymerase III^{ts}, DNA polymerase I^{-}) which is more suitable for studies of DNA synthesis in vitro than the single mutant strain U2355*pol III*^{ts} (DNA polymerase III^{ts}).

The results presented in Fig. 1 describe the synthesis of DNA in uninfected and ϕe-infected cells, in the presence and absence of HPUra.

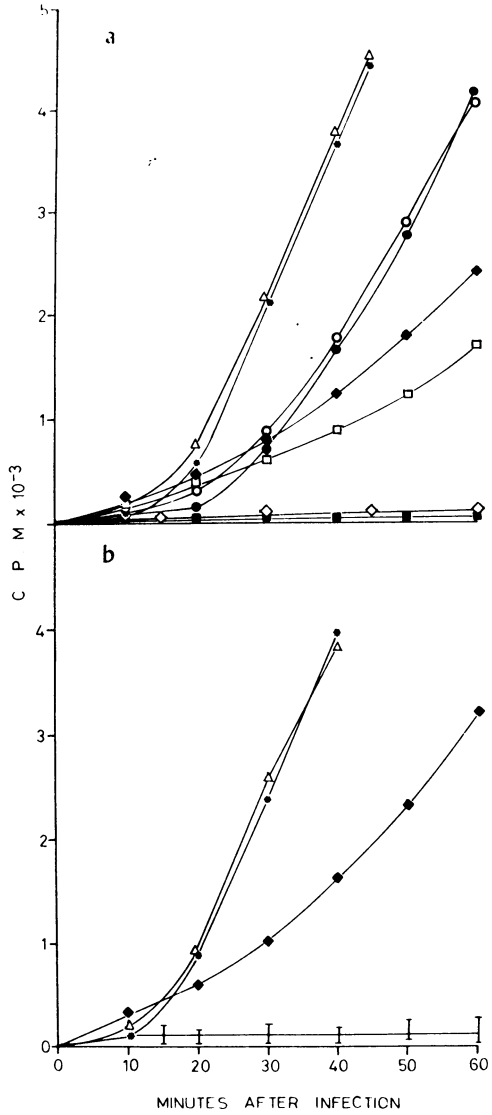

FIG. 1. *DNA synthesis in ϕe-infected cells at 37 and 48 C in presence and absence of HPUra. B. subtilis strains 2344pol III⁺ and 2355pol III^{ts} were grown at 37 C to 2 × 10⁸ cells per ml. One minute before infection, each culture was divided into eight portions, four of which are transferred to 48 C. HPUra (final concentration 0.15 mM) was then added where indicated. [2-¹⁴C]uracil (final concentration 5 μg/ml; 0.1 μCi/ml) and ϕe where indicated (MOI = 10) were added at zero time. Samples were removed and treated with alkali to hydrolyze labelled RNA. DNA was precipitated with trichloroacetic acid and radioactivity was determined. (a) 37 C; (b) 48 C. Symbols: ◆, 2344pol III⁺, uninfected; ◇, 2344pol III⁺, uninfected + HPUra; △, 2344 pol III⁺, infected with ϕe; ✳, 2344 pol III⁺, infected with ϕe + HPUra; □, 2355pol III^{ts}, uninfected; ■, 2355pol III^{ts}, uninfected + HPUra; ○, 2355pol III^{ts}, infected with ϕe; ●, 2355pol III^{ts}, infected with ϕe + HPUra. The vertical bars indicate that all the points (◇; □; ■; ○; ●) of the appropriate treatments fall within the ends of the bar.*

These results show that in ϕe-infected 2355*pol III*^{ts} cells, phage DNA is synthesized at 37 C but not at 48 C. On the other hand, in ϕe-infected 2344*pol III*⁺ cells—from which 2355*pol III*^{ts} was derived—phage DNA is synthesized both at 37 and 48 C. Even at 37 C the rate of phage DNA synthesis in ϕe-infected 2355*pol III*^{ts} cells is slower than in ϕe-infected 2344*pol III*⁺ cells. As expected, bacterial DNA synthesis is inhibited by HPUra and in 2355*pol III*^{ts} cells DNA synthesis is arrested at 48 C.

In ϕe-infected cells, DNA synthesis is resistant to HPUra from about 10 min after infection, at which time host DNA synthesis is arrested (18, 25). We conclude that the DNA synthesized under these conditions, from about 10 min after infection, is phage DNA. We therefore omitted in the following comparable experiments the HPUra treatment. In accordance with these findings, Table 1 shows that at 48 C virtually no mature phage is produced in ϕe-infected 2355*pol III*^{ts} cells. At the same temperature, the average burst size of ϕe grown in the parental strain 2344*pol III*⁺ is 25. At 37 C the average burst size of ϕe grown in both strains is about 100.

We isolated several spontaneous DNA polymerase III⁺ revertants from strain 2355*pol III*^{ts}. These revertants grow well at 48 C. They have lost the mutagenic character of their parent strain (3) and have an active DNA polymerase III both at 37 C and at 48 C. However these revertants still carry the DNA polymerase I⁻ mutation. The growth of ϕe in the various revertant clones isolated is normal both at 37 and 48 C. The data presented in Fig. 2 and in Table 1 were obtained with the revertant R2355*pol III*⁺. They clearly show that in ϕe-infected R2355 *pol III*⁺ cells phage DNA synthesis and progeny production are normal both at 37 and 48 C.

Requirement of protein synthesis for phage replication in a temperature shift down experiment. Figure 3 described the kinetics of DNA synthesis in cells infected with ϕe at 48 C and transferred to 37 C 21 min after infection, in presence or absence of chloramphenicol (CAP), an inhibitor of protein synthesis. These results show that in ϕe-infected 2355*pol III*^{ts}

TABLE 1. *Average burst size of φe grown at 37 or 48 C in strains 2344 pol III⁺, 2355 pol III^ts, and R2355 pol III⁺*

Host strain[a]	Burst size of φe grown at	
	37 C	48 C
2344 *pol III⁺*	100	25
2355 *pol III^ts*	95	<0.001
R2355 *pol III⁺*	110	32

[a] *B. subtilis* strains: 2344 *pol III⁺*, 2355 *pol III^ts* and R2355 *pol III⁺* were grown at 37 C to 3 × 10^8 cells per ml. The cells were infected at an MOI of 0.1 and shaken at 37 C for 10 min. The adsorption mixture was treated with anti-serum, diluted and plated for assay of phage (see Materials and Methods).

cells, phage DNA synthesis begins after the temperature shift only if protein synthesis is allowed to continue. Protein synthesis is also required for bacterial DNA synthesis in uninfected cells of strain 2355*pol III^ts* grown under similar conditions. On the other hand, in both uninfected and φe-infected 2344*pol III⁺* cells, DNA synthesis is not blocked at 48 C, and continues after the temperature shift to 37 C, even in presence of CAP.

Inactivation of DNA polymerase III leads to immediate arrest of phage DNA synthesis. The data presented above indicate that φe requires an active DNA polymerase III for its DNA replication. We have further investigated this requirement in order to find out whether DNA polymerase III is required only during part of the stage of phage DNA synthesis, or throughout the stage of phage replication. Figure 4 shows that the synthesis of phage DNA in φe-infected 2355*pol III^ts* cells, as well as that of bacterial DNA in uninfected cells, is arrested immediately upon transfer from 37 to 48 C. In the control experiment with uninfected and phage-infected 2344*pol III⁺* cells, DNA synthesis continues throughout the experiment.

DISCUSSION

B. subtilis has three distinct DNA polymerases (2, 8–10, 20). Several observations indicate that the product of the *polC* gene, DNA polymerase III, participates in the replication of the bacterial chromosome. Thus, mutants producing temperature-sensitive DNA polymerase III cannot synthesize their DNA at restrictive temperatures. Some of these mutants were found to be mutagenic (3, 9). A mutagenic DNA polymerase was found also in another system (30). Moreover, the drug HPUra which specifically blocks bacterial DNA synthesis in vivo (4) has been found to act by inhibiting DNA polymerase III (10, 17). The role of DNA polymerase II is not clear. DNA polymerase I participates in repair DNA synthesis (15).

Rutberg et al. (27) have found that DNA synthesis of the temperate *B. subtilis* phage

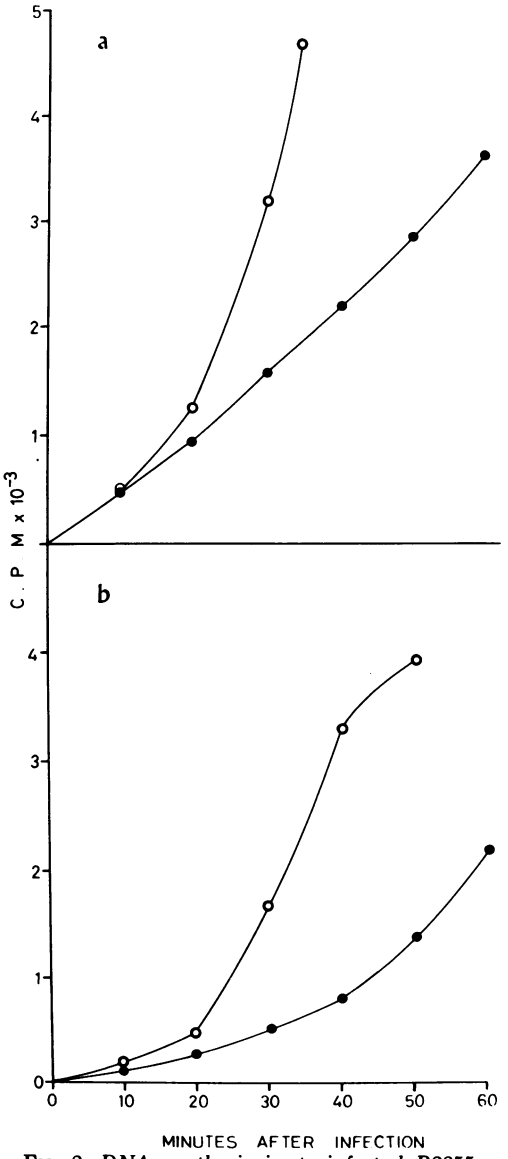

FIG. 2. *DNA synthesis in φe-infected R2355 pol III⁺ cells at 37 and 48 C. Strain R2355 pol III⁺ was grown at 37 C to 2 × 10^8 cells per ml. One minute before infection, the culture was divided into four portions, two of which were transferred to 48 C. [2-¹⁴C]uracil (final concentration 5 μg/ml; 0.1 μCi/ml) and φe where indicated (MOI = 10) were added at zero time. Samples were removed and radioactivity in DNA was determined as described in Fig. 1. (a) 37 C; (b) 48 C. Symbols: ●, R2355pol III⁺, uninfected; ○, R2355pol III⁺, infected with φe.*

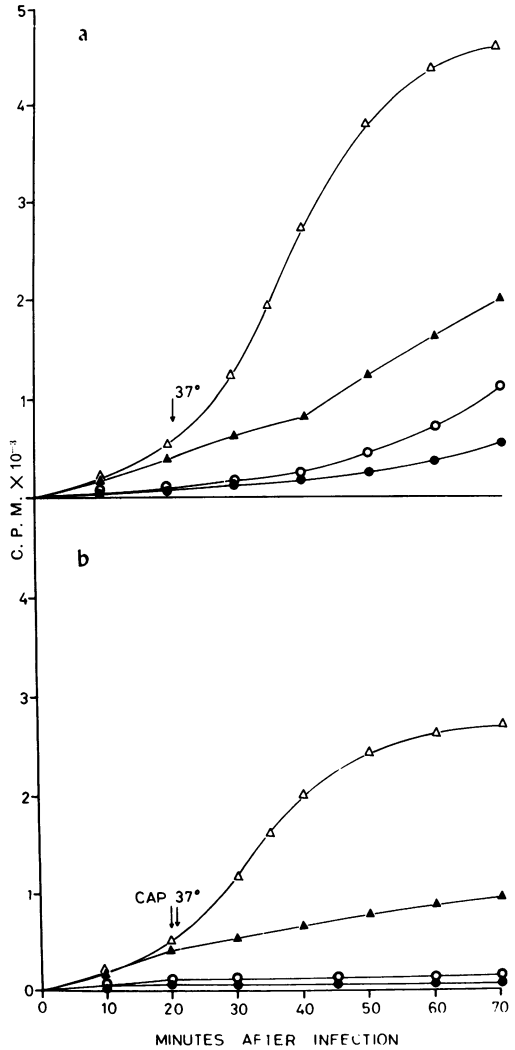

FIG. 3. *Effect of chloramphenicol on the synthesis of bacterial and phage DNA in a temperature-shift-down experiment. Strains 2344pol III⁺ and 2355pol III^{ts} were grown at 37 C to 2 × 10⁸ cells per ml. One minute before infection, each culture was divided into four portions, all of which were transferred to 48 C. [2-^{14}C]uracil (final concentration 5 μg/ml; 0.1 μCi/ml) and φe where indicated (MOI = 10) were added at zero time. Chloramphenicol (CAP, final concentration 100 μg/ml) was added 20 min after infection. One minute later, the shift to 37 C was performed. Samples were removed and radioactivity in DNA was determined as described in Fig. 1. (a) Shift to 37 C in absence of CAP; (b) shift to 37 C in presence of CAP. Symbols: ▲, 2344pol III⁺ uninfected; △, 2344pol III⁺ infected with φe; ●, 2355pol III^{ts} uninfected; ○, 2355pol III^{ts} infected with φe.*

φ105 is sensitive to HPUra in HPUra-sensitive host strains, but is resistant to the drug in HPUra-resistant mutant strains. Neville and Brown (20) and Cozzarelli and Low (5), have found that HPUra-resistant *B. subtilis* mutants produce a mutated DNA polymerase III which is insensitive to the drug. Therefore, the results obtained by Rutberg et al. indicate that host DNA polymerase III is required for φ105 DNA synthesis. DNA synthesis in the coliphages φX174 and M13 was also found to depend on host DNA polymerase III (7, 19).

The synthesis of DNA of the *B. subtilis* phages PBS2, SPO2, and SPO1 as well as that of φe is resistant to inhibition by HPUra. In cells infected with these phages, new DNA polymerase activities were detected (23, 27, and unpublished data, respectively). However, no new DNA polymerase activity has been found in extracts of φ105-infected cells (27). Rutberg et al. (27) suggested that all the *B. subtilis* phages which are capable of directing the synthesis of their DNA in the presence of HPUra induce the synthesis of a phage-specific DNA polymerase. Indeed, a new DNA polymerase was purified from phage SPO1-infected cells (33). The role of the new DNA polymerase which appears in cells infected with the phages SPO2, PBS2, SPO1, or φe is not yet known. The coliphages T4, T5, and T7 were also found to induce the synthesis of their own DNA polymerases. Genetic studies have shown that these enzymes are essential for the synthesis of phage DNA (6, 12, 31).

We were interested in understanding the mechanism whereby φe arrests host DNA synthesis several minutes after infection (16, 18, 24, 25). This phage induces on infection the synthesis of both an inhibitor of the host thymidylate synthetase and a deoxythymidinetriphosphate nucleotidohydrolase (dTTPase) (13, 24, 25). Previous studies have shown that depletion of the dTTP pool in φe-infected cells is not the only cause for host-DNA arrest by this phage. Upon infection with a dTTPase mutant phage, the arrest of host DNA synthesis takes place even in thymine requiring cells provided with exogenous thymine (18). Another φe-directed mechanism is known to be responsible for arresting the synthesis of host DNA. Mutants in which this mechanism does not operate (18), and consequently do not shut off host DNA synthesis, are designated as *bda*⁻ (for *b*acterial DNA arrest⁻). We have shown earlier (16) that in toluenized preparations of φe-infected cells host DNA synthesis is blocked in a manner which closely resembles DNA arrest in vivo. Similar (unpublished) results were obtained with lysates of φe-infected cells spread on cellophane disks (28).

It is conceivable that host DNA arrest is effected by an interaction between the *bda*-gene

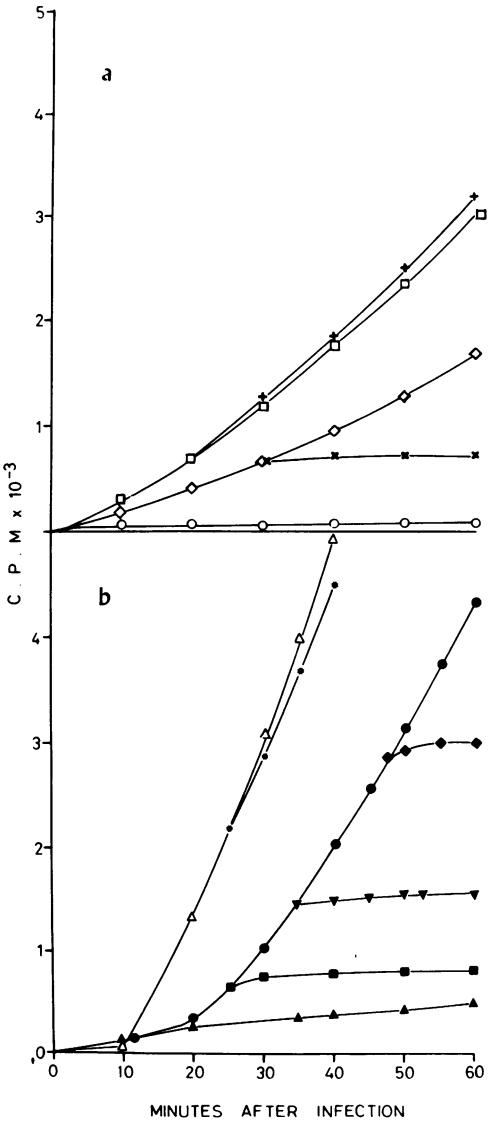

FIG. 4. *Kinetics of bacterial and phage DNA synthesis in a temperature-shift-up experiment. Strains 2344pol III⁺ and 2355pol III^ts were grown at 37 C to 2 × 10⁸ cells per ml. One minute before infection, each culture was divided into two portions. At zero time, [2-¹⁴C]uracil (final concentration 5 μg/ml; 0.1 μCi/ml) and φe where indicated (MOI = 10) were added. Portions were transferred from 37 to 48 C at different times after infection. Samples were removed and radioactivity in DNA was determined as described in Fig. 1. (a) Uninfected cells, symbols: □, 2344pol III⁺ incubated at 37 C; +, 2344pol III⁺ transferred to 48 C at 30 min; ◇, 2355pol III^ts incubated at 37 C; ○, 2355pol III^ts transferred to 48 C at 0 min; ×, 2355pol III^ts transferred to 48 C at 30 min. (b) φe-Infected cells, symbols: △, 2344 pol III⁺ incubated at 37 C; ✳, 2344pol III⁺ transferred to 48 C at 25 min; ●, 2355pol III^ts incubated at 37 C; ▲, 2355pol III^ts transferred to 48 C at 10 min; ■, 2355pol III^ts transferred to 48 C at 25 min; ▼, 2355pol III^ts transferred to 48 C at 35 min; ◆, 2355pol III^ts transferred to 48 C at 45 min.*

product and the host DNA polymerase III.

We investigated this possibility by measuring the growth of φe in a thermosensitive DNA polymerase III host. It is evident that no φe DNA is synthesized in cells of strain 2355*pol III^ts* at 48 C. Accordingly, no progeny phage is produced at this temperature. Under similar conditions, both DNA synthesis and phage production were normal in φe-infected cells of strain 2344*pol III⁺* (from which 2355*pol III^ts* has been derived) and in φe-infected cells of the revertant strain R2355*pol III⁺*. These results indicate that the failure of φe-infected cells of strain 2355*pol III^ts* to support the synthesis of phage DNA is due exclusively to the inactivation of the thermolabile DNA polymerase III. We have obtained similar results with phage SPO1 (data not shown).

Inhibition of bacterial DNA synthesis per se cannot account for the arrest of phage-DNA synthesis. This is borne out by the finding that phage DNA synthesis is normal in thymine-requiring host cells that are starved for thymine (unpublished data), as well as in HPUra-treated cells (18). The temperature-shift-down experiment reported here indicates that, in φe-infected 2355*pol III^ts* cells, the thermolabile DNA polymerase III is irreversibly inactivated at 48 C and has to be synthesized de novo before phage DNA synthesis can begin. This experiment also shows that the inability of φe to direct the synthesis of its own DNA in 2355*pol III^ts* cells at 48 C is not due to the high temperature per se, but to the inactivation of DNA polymerase III. The immediate arrest of phage-DNA synthesis in φe-infected 2355*pol III^ts* cells upon transfer from 37 to 48 C indicates that DNA polymerase III is required for phage-DNA synthesis throughout the stage of phage replication.

The requirement for DNA polymerase III by phage φe is unexpected in view of the resistance of phage-DNA synthesis to HPUra. The explanation of these conflicting data may lie with the phage-induced bacterial-DNA-arrest protein which is responsible for host-DNA arrest. We propose that this protein modified DNA polymerase III in such a way that the enzyme stops replicating bacterial DNA and starts to replicate phage DNA. The same modification should also account for the loss of sensitivity to HPUra by the enzyme. The connection between DNA polymerase III and the new DNA polymerase which appears in phage-infected cells (33) is not clear.

We are now studying in vitro the interaction between a purified bacterial-DNA-arrest protein and DNA polymerase III.

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LITERATURE CITED

1. Aposhian, H. V. 1965. A dTMPase found after infection with phage SP5C. Biochem. Biophys. Res. Commun. **18**:230-235.
2. Bazill, G. W., and J. D. Gross. 1972. Effert of 6-(p-Hydroxyphenyl)-azo uracil on *B. subtilis* DNA polymerases. Nature N. Biol. **240**:82-83.
3. Bazill, G. W., and J. D. Gross. 1973. Mutagenic DNA polymerase in *B. subtilis*. Nature N. Biol. **243**:240-243.
4. Brown, N. C. 1971. Inhibition of bacterial DNA replication by 6-(p-Hydroxyphenylazo)-uracil: differential effect on repair and semi-conservative synthesis in *Bacillus subtilis*. J. Mol. Biol. **59**:1-16.
5. Cozzarelli, N. R., and R. L. Low. 1973. Mutational alteration of *Bacillus subtilis* DNA polymerase III to hydroxyphenylazo-pyrimidine resistance: polymerase III is necessary for DNA replication. Biochem. Biophys. Res. Commun. **51**:151-157.
6. DeWaard, A., A. V. Paul, and I. R. Lehman. 1965. The structural gene for deoxyribonucleic acid polymerase in bacteriophages T4 and T5. Proc. Nat. Acad. Sci. U. S. A. **54**:1241-1248.
7. Dumas, L. B., and C. A. Miller. 1973. Replication of bacteriophage ϕX174 DNA in a temperature sensitive dnaE mutant of *Escherichia coli* C. J. Virol. **11**:848-855.
8. Ganesan, A. T., C. O. Yehle, and C. C. Yu. 1973. DNA replication in a polymerase I deficient mutant and the identification of DNA polymerase II and III in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. **50**:155-163.
9. Gass, K. B., and N.R. Cozzarelli. 1973. Further genetic and enzymological characterization of the three *Bacillus subtilis* deoxyribonucleic acid polymerases. J. Biol. Chem. **248**:7688-7700.
10. Gass, K. B., R. L. Low, and N. R. Cozzarelli. 1973. Inhibition of a DNA polymerase from *Bacillus subtilis* by hydroxyphenylazopyrimidines. Proc. Nat. Acad. Sci. U. S. A. **70**:103-107.
11. Green, D. M. 1964. Infectivity of DNA isolated from *Bacillus subtilis* bacteriophage, SP82. J. Mol. Biol. **10**:438-451.
12. Grippo, P., and C. C. Richardson. 1971. Deoxyribonucleic acid polymerase of bacteriophage T7. J. Biol. Chem. **246**:6867-6873.
13. Haslam, E. A., D. H. Roscoe, and R. G. Tucker. 1967. Inhibition of thymidylate synthetase in bacteriophage infected *Bacillus subtilis*. Biochim. Biophys. Acta **134**:312-326.
14. Kallen, R. G., M. Simon, and J. Marmur. 1962. The occurence of a new pyrimidine base replacing thymine in a bacteriophage DNA: 5-Hydroxymethyluracil. J. Mol. Biol. **5**:248-250.
15. Laipis, P. J., and A. T. Ganesan. 1972. A deoxyribonucleic acid polymerase I-deficient mutant of *Bacillus subtilis*. J. Biol. Chem. **247**:5867-5871.
16. Lavi, U., and M. Marcus. 1972. Arrest of host DNA synthesis in *Bacillus subtilis* infected with phage ϕe. Virology **49**:668-674.
17. Mackenzie, J. M., M. M. Neville, G. E. Wright, and N. C. Brown. 1973. Hydroxyphenylazopyrimidines: characterization of the active forms and their inhibitory action on a DNA polymerase from *Bacillus subtilis*. Proc. Nat. Acad. Sci. U. S. A. **70**:512-516.
18. Marcus, M., and M. C. Newlon. 1971. Control of DNA synthesis in *Bacillus subtilis* by phage ϕe. Virology **44**:83-93.
19. Mitra, S., and D. R. Stallions. 1973. Role of dna genes of *Escherichia coli* in M13 phage replication. Virology **52**:417-424.
20. Neville, M. M., and N. C. Brown. 1972. Inhibition of a discrete bacterial DNA polymerase by 6-(p-Hydroxyphenylazo)-uracil and 6-(p-Hydroxyphenylazo)isocytosine. Nature N. Biol. **240**:80-82.
21. Okuho, S., B. Strauss, and M. Stadolsky. 1964. The possible role of recombination in the infection of competent *Bacillus subtilis* by bacteriophage deoxyribonucleic acid. Virology **24**:552-562.
22. O'Sullivan, A., and N. Sueoka. 1967. Sequential replication of the *Bacillus subtilis* chromosome. IV. Genetic mapping by density transfer experiment. J. Mol. Biol. **27**:349-368.
23. Price, A., and S. J. Cook. 1972. New deoxyribonucleic acid polymerase induced by *Bacillus subtilis* bacteriophage PBS2. J. Virol. **9**:602-610.
24. Roscoe, D. H. 1969. Thymidine triphosphate nucleotidohydrolase. A phage induced enzyme in *B. subtilis*. Virology **38**:520-523.
25. Roscoe, D. H. 1969. Synthesis of DNA in phage-infected *Bacillus subtilis*. Virology **38**:527-537.
26. Roscoe, D. H., and R. G. Tucker. 1966. The biosynthesis of 5-Hydroxymethyldeoxyuridylic acid in bacteriophage-infected *Bacillus subtilis*. Virology **29**:157-166.
27. Rutberg, L., R. W. Armentrout, and J. Jonasson. 1972. Unrelatedness of temperate *Bacillus subtilis* bacteriophage SPO2 and ϕ105, J. Virol. **9**:732-737.
28. Schaller, H., B. Otto, V. Nuslein, J. Huf, R. Herrman, and F. Bonhoeffer. 1972. Deoxyribonucleic acid replication *in vitro*. J. Mol. Biol. **63**:183-200.
29. Sonenshein, A. L., and D. H. Roscoe. 1969. The course of phage ϕe infection in sporulating cells of *Bacillus subtilis* strain 3610. Virology **39**:265-276.
30. Speyer, J. F. 1965. Mutagenic-DNA polymerase. Biochem. Biophys. Res. Commun. **21**:6-8.
31. Warner, H. R., and J. E. Barnes. 1966. Deoxyribonucleic acid synthesis in *Escherichia coli* infected with some deoxyribonucleic acid polymerase-less mutants of bacteriophage T4. Virology **28**:100-107.
32. Yehle, C. O., and A. T. Ganesan. 1972. Deoxyribonucleic acid synthesis in bacteriophage SP01-infected *Bacillus subtilis*. J. Virol. **9**:263-272.
33. Yehle, C. O., and A. T. Ganesan. 1973. Deoxyribonucleic acid synthesis in bacteriophage SPO1-infected *Bacillus subtilis*. J. Biol. Chem. **248**:7456-7463.