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SYNTHESIS OF DNA CONTAINING URACIL

(OR 5-HYDROXYMETHYLURACIL)

DURING BACTERIOPHAGE INFECTION OF Bacillus subtilis

COMPREHENSIVE REPORT (3 year)

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DISCLAIMER

"Synthesis of DNA Containing Uracil (or 5-Hydroxymethyluracil) during Bacteriophage Infection of Bacillus subtilis"

ABSTRACT

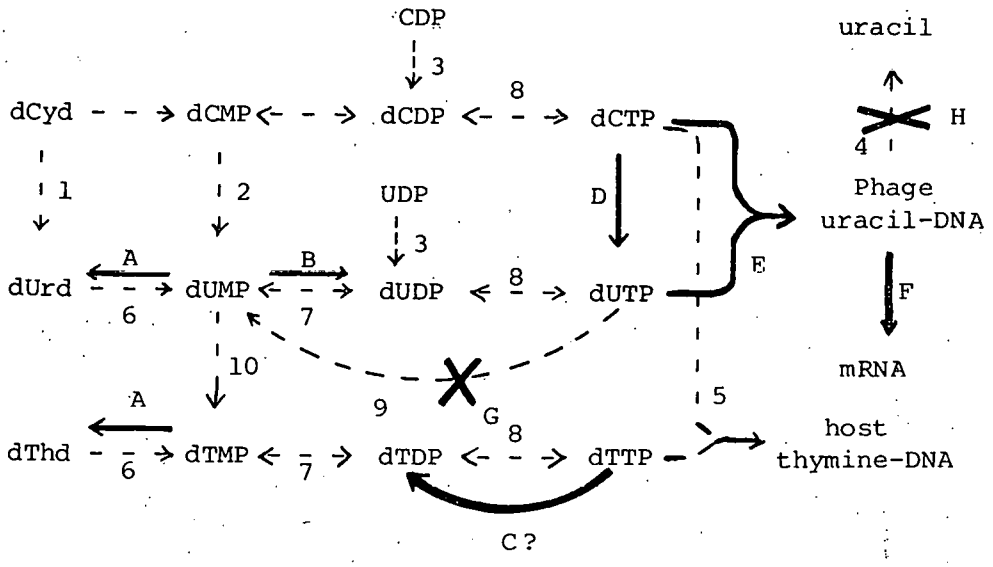
The infection of Bacillus subtilis (whose DNA contains thymine) by viruses whose DNA contains uracil (PBS2 phage) or 5-hydroxymethyluracil (ϕe phage) instead of thymine has been studied. The goal was to learn how and why these unusual viruses make DNA containing unusual bases and to gain insight into why thymine evolved for most natural DNAs.

We discovered a new PBS2 phage-induced DNA polymerase, RNA polymerase, and an inhibitor-protein which blocks the host's dUTPase. We have characterized the PBS2-induced dTMPase, dUMP kinase, dCTP deaminase, and an inhibition of the host's uracil-DNA N-glycosidase. We have also designed separation methods to determine changes in the deoxyribonucleotide pools after PBS2 infection. Thus, the roles of PBS2 enzymes and inhibitors as well as drugs and mutants can be studied in vivo, to prove the mechanism or the need for uracil-DNA synthesis by PBS2 phage.

The structure and function of the dTTPase-dUTPase induced by ϕe phage have been determined. The enzyme's role is to exclude thymine from the ϕe hydroxymethyluracil-DNA. We have also begun experiments to determine the mechanism of the cytostatic action of 5-hydroxymethyldeoxyuridine [a component of ϕe DNA] on animal cells.

INTRODUCTION

Almost all natural DNA contains thymine. However, there is one Bacillus subtilis bacteriophage, PBS2 (and its close relatives: PBS1, 3NT, AR9, etc.) which has uracil in its DNA (1,2) instead of thymine (which is 5-methyluracil). We have been studying the biosynthesis and function of phage PBS2 uracil-DNA. Our work has been centered around the metabolic pathway shown below, which is based on the available information for B. subtilis (see below, and refs. 3 and 4) and by analogy to Escherichia coli enzymes and phage T4 infection (3,5). I proposed this scheme for pyrimidine deoxyribonucleotide metabolism in B. subtilis for thymine-DNA synthesis, as modified by phage PBS2 for uracil-DNA synthesis:



B. subtilis enzymes

1. deoxycytidine deaminase
2. dCMP deaminase
3. NDP reductase
4. uracil-DNA N-glycosidase
5. DNA polymerases I, II, and III
6. thymidine (deoxyuridine) kinase
7. dTMP (dUMP) kinase
8. NDP/dNDP kinase
9. dUTPase
10. dTMP synthetase

PBS2 phage-induced

A. dTMPase
B. dUMP kinase
C. dTTPase (?)
D. dCTP deaminase
E. DNA polymerase
F. RNA polymerase
G. dUTPase inhibitor
H. N-glycosidase inhibitor

A similar scheme can be drawn for B. subtilis infection by ϕe phage, one of a large class of B. subtilis viruses whose DNA contains 5-hydroxymethyluracil instead of thymine (4,6). This virus induces activities which hydrolyze dUTP to dUMP and PPi and dTTP to dTMP and PPi, apparently to exclude uracil and thymine from the ϕe hydroxymethyluracil-DNA (6).

ORIGINALLY STATED OBJECTIVES

PBS2 Phage with Uracil instead of Thymine in its DNA

"I propose to study pyrimidine deoxyribonucleotide metabolism during PBS2 infection to learn how uracil-DNA is synthesized and how it functions in a cell which normally makes thymine-DNA. I hope to determine whether the substitution of uracil for thymine in DNA alters the biological properties of the DNA, and whether there is a functional significance to the observation that almost all other known DNA species contain thymine." [1970]

"We will assay crude extracts of uninfected and PBS2-infected B. subtilis cells for the enzymatic activities described in the above scheme. This information will help us to recognize the probable pathways of deoxyribonucleotide metabolism for the synthesis of host thymine-DNA and phage uracil-DNA. We will purify the most important host and phage-induced enzymes and characterize them for their specificity and their kinetic and physical properties. We will also determine the effects of various inhibitors on DNA synthesis and of host and phage mutations on the ability of PBS2-infected cells to make uracil-DNA. Using appropriate mutants in the host and phage-induced enzymes, we can examine the changes in nucleotide metabolism to prove the precise role of these enzymes during infection. We hope to determine whether PBS2 DNA can be biologically functional whether it contains uracil or thymine, or whether a unique function exists for the usual uracil in PBS2 DNA." [1973]

ϕe Phage with 5-Hydroxymethyluracil instead of Thymine in its DNA

"We will purify and characterize physically and kinetically the ϕe-induced dTTPase and dUTPase to prove whether a single nucleotidohydrolase possesses both enzymatic activities. We will prove that certain ϕe mutants have an altered nucleotidohydrolase protein structure for this enzyme. We will then use these mutants with defective dTTPase and dUTPase activities to determine their function during ϕe infection. We want to know whether these activities are essential for excluding thymine and uracil from ϕe DNA. Furthermore, we intend to discover whether dHMU is essential for the function of ϕe DNA, or whether this phage is infective when its DNA contains thymine (or uracil) instead of hydroxymethyluracil." [1970]

MAIN RESEARCH ACCOMPLISHMENTS AND NEW OBJECTIVES

PBS2 Phage with Uracil instead of Thymine in its DNA

PBS2 dTMPase

Kahan's abstract (7) briefly described a PBS2 phage-induced dTMPase, which produced thymidine and Pi (enzyme #A above), and was

thought to help exclude thymine from PBS2 DNA. We purified and characterized the dTMPase (8). The data suggested a native molecular weight of 40,000, with subunits likely. Its specificity was studied using 35 natural or synthesized substrate analogues. Parallel induction kinetics, pH dependence, heat and trypsin inactivation, sulfhydryl reagent and fluoride inhibition, metal ion effects, $K_m = K_i$, and co-purification proved that one enzyme had both dTMPase (K_m = 10 μM) and dUMPase (K_m = 1000 μM) activities. We suggested that this PBS2 phosphohydrolase functions to exclude thymine from PBS2 DNA, by eliminating the substrate (dUMP) and the product (dTMP) of the B. subtilis dTMP synthetase (enzyme #10).

PBS2 dUMP kinase

Kahan's abstract (7) also noted the appearance of a PBS2-induced dUMP kinase: dUMP + ATP → dUDP (and dUTP) + ADP (enzyme #B). When we discovered a PBS2-induced inhibitor of the B. subtilis dUTPase (protein #G and enzyme #9, respectively), we wondered whether this inhibition of the "reverse" reaction [dUTP —x→ dUMP + PPi] might explain the apparent "induction of dUMP kinase" activity.

As described in the 1976 Progress Report, we have partially purified the PBS2 dUMP kinase, separating it from the host dUTPase, the phage dUTPase inhibitor, and the low level of host dUMP (dTMP) kinase (enzyme #7). We plan to characterize this phage-induced dUMP kinase, determining its K_m and its specificity for dUMP versus other nucleotides. We want to know whether it has the properties expected for an enzyme whose function is to provide dUDP and dUTP as precursors to PBS2 uracil-DNA.

PBS2 dTTPase (?)

Tomita and Takahashi (personal communication) have preliminary evidence that there is a transient 2-fold increase in a low-level dTTPase (enzyme #C) activity producing dTDP after PBS2 infection. Our assays do show dTTP hydrolysis, and we intend to investigate ourselves whether a new phage enzyme is really induced.

PBS2 dCTP deaminase

Tomita and Takahashi (9) described the induction, partial purification, and specificity of a PBS2 dCTP deaminase, which produced dUTP and NH_3 (enzyme #D). They claimed that the K_m for dCTP was 360 μM with linear saturation kinetics; but this was inconsistent with their time course of reaction. We observed a sigmoidal dCTP saturation curve, with an extrapolated K_m of 50 to 100 μM for dCTP with a Hill number of 1.20; this suggests positive cooperativity in dCTP binding sites (10). The enzyme has a molecular weight of about 125,000 as judged by gel filtration and sucrose gradient analysis. It is inhibited by sulfhydryl reagents and by EDTA (and is stimulated by Mg^{2+}).

While Takahashi (9) and I (10) both observed strong inhibition of the PBS2 dCTP deaminase by dTTP, it is unlikely that this occurs *in vivo*. We believe that dTTP would be eliminated from PBS2-infected cells (see above); thus, this sensitivity to dTTP may reflect the evolution of this enzyme from a cellular deaminase sensitive to dTTP. But we were pleased to discover that dUTP inhibits the PBS2 dCTP deaminase, although dUTP is 5-fold less potent than dTTP. Thus, product inhibition by dUTP (which can be overcome by high dCTP concentrations) would seem to provide the necessary control on the irreversible PBS2 dCTP deaminase (10), allowing balanced levels of both dCTP and dUTP to be available for PBS2 DNA synthesis.

PBS2 DNA polymerase

We discovered a large increase in DNA polymerase (enzyme #E) activity after PBS2 infection of wild-type or *polA* (DNA polymerase I-deficient) *B. subtilis* cells (2). The enzyme required Mg^{2+} and all 4 deoxyribonucleoside triphosphates; it was 70% inhibited by *p*-hydroxymercuribenzoate. The PBS2-induced DNA polymerase seemed to prefer denatured DNA over native DNA, PBS2 uracil-DNA over salmon thymine-DNA, and dUTP over dTTP [all at high concentrations]. The major *B. subtilis* DNA polymerase (*polA*$^+$-coded DNA polymerase I) was insensitive to sulfhydryl reagents and preferred dTTP to dUTP.

My graduate student, Ron Hitzeman, has spent several years working out a purification scheme for the PBS2 DNA polymerase (see 1976 Progress Report). Although there are problems with instability, we can now separate the phage enzyme from the host DNA polymerase I, II, and III (11). We routinely use a *polA* host, so that polymerase I is absent; the PBS2 DNA polymerase assay using single-stranded DNA then detects little host activity (less than 1%). Using DNase-degraded DNA and 70 mM (high level) $MgCl_2$ gives 10-fold more activity for phage DNA polymerase, but also detects considerable residual host polymerases. The *B. subtilis* DNA polymerase II and III assay at 7 mM $MgCl_2$ on activated DNA does not detect PBS2 DNA polymerase. Furthermore, the *polC*$^+$-coded host polymerase III can be blocked by N-ethylmaleimide (11) or 6-(*p*-hydroxyphenylazo)-uracil [HPUra] (11,12). We find that PBS2 DNA polymerase *in vitro* and PBS2 DNA synthesis *in vivo* are unaffected by HPUra (13,14).

We plan to characterize the PBS2 DNA polymerase for its size, metal ion and pH dependence, K_m for dUTP versus dTTP, K_m for uracil-DNA versus thymine-DNA, nuclease activity, etc. We want to determine whether its properties are consistent with the selective replication of PBS2 DNA in infected cells (15).

The resistance of PBS2 replication to HPUra (see above) and the normal induction of stable PBS2 DNA polymerase in *B. subtilis* F25

(a polC mutant with a labile DNA polymerase III; see ref. 11) indicates that host polymerase III is not used in PBS2 infection. However, results with pol III^{ts} mutants (see 1976 Progress Report) suggest that PBS2 DNA synthesis may involve the host's DNA polymerase III, at least under certain conditions:

a) while the rate of PBS2 DNA synthesis is normal at 50° versus 37° in a polA strain, Marcus' polA polC^{ts}(mut-1) strain #2355 (16) allows PBS2 DNA synthesis only at 37° and not at 50°;

b) while the rate of PBS2 DNA synthesis is unaffected by HPUra in a polA cell at 37° and 50°, Cozaraelli's polA polC^{ts}(mut-1) strain #BD317 allows HPUra-resistant PBS2 DNA synthesis at 37° -- but at 50°, PBS2 DNA synthesis can be blocked by HPUra.

However, both mutant and wild-type strains give no phage burst above 47°, and many cells remain uninfected and in chains in the mutant cultures. We are now continuing these studies to determine whether B. subtilis DNA polymerase III has a role in PBS2 replication.

Inhibitors of macromolecular synthesis

We studied several inhibitors known to block the synthesis of macromolecules in B. subtilis for their effects on PBS2 phage reproduction:

a) HPUra, which blocks host DNA synthesis by competing with dGTP for DNA polymerase III (see above, and refs. 11 and 12), did not affect PBS2 infection (13).

b) nalidixic acid, which blocks host DNA synthesis at low concentrations by an unknown mechanism, has little effect on PBS2 infection. At high concentrations (50 µg/ml), the PBS2 burst was inhibited more severely than the levels of PBS2 DNA synthesis (17).

c) hydroxyurea, which inhibits host DNA synthesis by blocking ribonucleoside diphosphate reductase (enzyme #3), also blocked PBS2 DNA synthesis (17).

d) phleomycin, which inhibits bacterial DNA synthesis, did not affect PBS2 enzyme production. However, PBS2 DNA synthesis was greatly reduced, the parental DNA was degraded, and late virion protein synthesis was eliminated (as judged by dodecyl-sulfate slab-gel electrophoresis/^{14}C-autoradiographic analysis (14).

e) actinomycin D and lucanthone (which block host RNA synthesis by binding to the DNA template) and chloramphenicol (which blocks

bacterial protein synthesis on the ribosome) also prevented the induction of PBS2 proteins (2,8,15,18,19).

f) rifampicin, streptovaricin, and streptolydigin (which block B. subtilis RNA synthesis by binding to RNA polymerase) were discovered by us to have no effect on PBS2 reproduction (15). Even huge amounts of rifampicin (100 µg/ml) could be added before infection without reducing PBS2 enzyme induction and burst size. Rima and Takahashi (20) independently found the same thing. We suggested that PBS2 phage induced its own rifampicin-resistant RNA polymerase; perhaps PBS2 even injected this RNA polymerase from the virion with the DNA into the cell, so that phage mRNAs could be made in rifampicin without using the host's rifampicin-sensitive RNA polymerase. In fact, PBS2 DNA is inactive as a template for B. subtilis RNA polymerase (20).

PBS2 RNA polymerase

Indeed, we found that PBS2 induced a new RNA polymerase (enzyme #F) activity after infection (18). It was specific for PBS2 DNA as a template; the enzyme was resistant to rifampicin, 8 rifamycin derivatives, streptovaricin, and streptolydigin. The activity was inhibited by actinomycin D and lucanthone. These in vitro results exactly paralleled the in vivo effects on PBS2 reproduction (see above). Clark et al. (21) independently discovered and purified the PBS2 RNA polymerase, showing it to possess 4 subunits all different in molecular weight from the 4 B. subtilis RNA polymerase subunits. It is still not clear whether the virion carries this RNA polymerase (or some of its subunits) and injects it with the DNA on infection.

PBS2 inhibitor of host dUTPase

We reported (22) that B. subtilis cells contain a dUTPase activity which hydrolyzes dUTP to dUMP and PPi (enzyme #9). It has an apparent molecular weight of 48,000 daltons [30 A Stokes radius; 3.5 S on sucrose gradients] (19). Its isoelectric point is at pH 4.1. It is stimulated by Mg^{2+}, inhibited by EDTA, specific for dUTP (K_m = 2 µM) among all nucleotides tested, and has a broad pH optimum around pH 8.5. It is believed to help exclude dUTP from cellular DNA (3,5,19,23).

We then discovered (19) in PBS2-infected cells an induced protein (protein #G) which inhibits the host dUTPase activity -- as predicted from the need for the PBS2 DNA polymerase to utilize dUTP for uracil-DNA synthesis. This phage-induced dUTPase inhibitor can be separated from residual dUTPase activity by Sephadex column chromatography. The inhibitor is heat-labile, trypsin-sensitive, sulfhydryl reagent-insensitive, and has an apparent molecular size of 83,000 daltons (probably a dimer).

The inhibitor acts reversibly, since reaction mixtures containing uninfected dUTPase and PBS2-induced inhibitor can be subjected to gel filtration to separate active enzyme (with the same K_m) and active inhibitor. The inhibitor appears to form a 4.9 S complex with the dUTPase itself and does not alter the substrate nor the product of the dUTPase reaction.

The inhibitor functions best in vitro at an assay pH of 6 to 7, with its action declining to a minimum at pH 9.7. Likewise, the PBS2 dTMPase (enzyme #A), believed to help exclude dTTP as a substrate for PBS2 DNA polymerase, has a similar pH-activity profile (8). Thus, we thought it might be possible in vivo to raise the pH of the medium from 7 to 9 and perhaps thereby prevent the action of the inhibitor and the dTMPase. This might allow degradation of dUTP and synthesis of dTTP (see scheme above), stopping PBS2 uracil-DNA synthesis and perhaps allowing thymine-DNA synthesis, even in the presence of HPUra.

In fact, we observed that there is a sharp drop in PBS2 burst size as the medium's pH is increased from pH 8 to pH 8.9 (19). [The eclipse of input phage, the time of onset of progeny phage production, and the specific activity of several PBS2 enzymes tested were all normal at pH 8.9.] Furthermore, a parallel fall in the amount of DNA synthesis occurred as the pH was raised. Most interestingly, the newly synthesized DNA had an altered thymine/uracil base ratio. The ratio was less than 0.03 in pH 8 infections (only uracil in the phage DNA), and it increased gradually with increasing pH to a ratio at pH 8.9 of 1.0 (equal amounts of uracil and thymine in the new DNA). These data support the proposal that infection at high pH prevents the PBS2 dUTPase inhibitor from functioning, thus allowing dUTPase to degrade dUTP for dTTP synthesis, so that thymine replaces some of the uracil in newly synthesized DNA. By these and other chemical or genetic methods, we hope to determine whether PBS2 DNA can function with thymine versus uracil in it.

PBS2 inhibitor of host uracil-DNA N-glycosidase

Last summer Friedberg (24) and Takahashi (25) described an enzyme(s) in B. subtilis extracts which specifically degraded uracil-containing PBS2 DNA (enzyme #4). DNA containing thymine, 5-hydroxymethyluracil, or 5-bromouracil was not attacked. Friedberg (24) reported that free uracil was the only product made in the presence of EDTA, making the enzyme an N-glycosidase. Takahashi (25) indicated that oligonucleotides and deoxyuridine were produced in $CaCl_2$, suggesting the enzyme was an endonuclease [although no proof of uracil versus deoxyuridine was shown nor mentioned]. This enzyme is believed to help to exclude uracil from normal thymine-DNA. Thus, it was predicted and confirmed that PBS2 phage should completely inhibit

this enzyme -- by 4 minutes after infection in Friedberg!s assays (24) and by 20 minutes after infection in Takahashi's assays (25). Takahashi proved that the inhibitor was a phage-induced protein of 15,000 molecular weight (protein #H).

We set up Friedberg's and Takahashi's assay conditions for testing on our own extracts. We found that both assays gave similar results; the degradative enzyme(s) attacking uracil-DNA were inhibited from 3 to 7 minutes after infection. This period was substantially earlier than the appearance of any other PBS2 enzyme (see data in 1976 Progress Report). Furthermore, we could show by using mixed extracts that the synthesis of the inhibitor continued, so that more inhibitor was present at 30 (than 20) than 10 minutes after infection. We are pursuing the characterization of the enzyme(s) and inhibitor(s), to resolve the discrepancies between 2 published reports (24,25) and to show that this inhibitor is another unique, PBS2-induced protein.

Experiments (see 1976 Progress Report) using phage with [^{3}H]-uracil-labelled DNA suggested that the uracil-DNA N-glycosidase (or nuclease) was not attacking injected PBS2 DNA early in infection. Perhaps the PBS2 inhibitor of the N-glycosidase is injected with the DNA (as proposed for the PBS2 RNA polymerase above).

Deoxyribonucleotide pool separations

The above metabolic scheme predicts large changes in the deoxyribonucleotide pools of B. subtilis after PBS2 phage infection. First, dTTP (plus dTTP and dTMP) as precursors to thymine-DNA should be much reduced; perhaps they are excreted from the cell as thymidine by action of the PBS2 dTMPase (enzyme #A). Second, dUTP [which has never been detected in vivo (26)] should accumulate for the synthesis of uracil-DNA by the PBS2 DNA polymerase (enzyme #E). This dUTP is likely to be made by action of the PBS2 dCTP deaminase (enzyme #D), the host's dCMP deaminase (enzyme #2) plus the PBS2 dUMP kinase (enzyme #B), and the host's ribonucleotide reductase (enzyme #3) plus the host's nucleoside diphosphate kinase (enzyme #8). It is not clear whether the high K_m-dUMPase (enzyme #A, which is also the PBS2 low K_m-dTMPase) would remove dUMP from the cell as deoxyuridine. Nor is it proven whether the host's dTMP synthetase (enzyme #10) is shut off after PBS2 infection to prevent thymidine-nucleotide synthesis.

As described in the 1976 Progress Report, my graduate student, Mr. Hitzeman, has developed a 2-dimensional thin-layer cellulose chromatography system which resolved dCTP, dUTP, and dTTP among other ribo- and deoxyribonucleoside mono-, di-, and triphosphates. We are now using this system to separate nucleotides extracted from uninfected

and PBS2-infected B. subtilis after labelling all pyrimidine compounds with [6-^{3}H]uracil. This method should prove that the pathways predicted above do occur and are altered as shown after PBS2 infection. This will give us new insight into the functions of the PBS2-induced proteins.

By use of these and other enzymatic, chemical, drug, and genetic techniques, we hope to determine how and why B. subtilis phage PBS2 makes DNA containing uracil instead of thymine. We trust that the information will help us to understand why thymine evolved for almost all other DNAs.

ϕe Phage with 5-Hydroxymethyluracil instead of Thymine in its DNA

ϕe dTTPase-dUTPase

We have isolated and characterized the nucleotidohydrolase induced by phage ϕe during infection of B. subtilis (6). We found that it is a single enzyme, membrane-bound but solubilizable, which hydrolyzes dTTP to dTMP and dUTP to dUMP [K_m's are 400 μM and 11 μM, respectively], with a V_{max} ratio of 1.3 for dTTP/dUTP (22). This enzyme was believed to help exclude thymine and uracil from the ϕe hydroxymethyluracil-DNA.

We have confirmed the observation (27) that ϕe mutants with only 2 to 10% of normal dTTPase-dUTPase activity make viable phage with 10% thymine substitution for hydroxymethyluracil. We have also found that such mutants have no uracil (less than 2% of hydroxymethyluracil) in their DNA (23). Thus, we do not know whether the ϕe dTTPase-dUTPase is essential, which of its 2 activities is more important, and whether hydroxymethyluracil is absolutely required in ϕe DNA. However, totally thymine-DNA would probably be stable in infected cells, since ϕe does not degrade the host thymine-DNA (6).

It would be useful in continuing these studies to have a ϕe double mutant, lacking both dTTPase-dUTPase and dUMP hydroxymethylase enzymes, so that we could eliminate hydroxymethyluracil-DNA synthesis and allow only thymine- (or uracil-)DNA synthesis. [We have already found ts dUMP hydroxymethylase mutants for the related hydroxymethyluracil-DNA phage SP82G (28).] Then we could test whether totally-thymine ϕe DNA is functional, or whether the hydroxymethyluracil in ϕe DNA is essential. My former graduate student, Dr. Linda Dunham, is continuing these studies in her faculty research at the University of Nevada.

Hydroxymethyldeoxyuridine and animal cells

With our expertise in analysis of unusual nucleosides, enzymes, and DNA in the hydroxymethyluracil-DNA phage system, we have begun a

new project on animal cells. We are studying the mechanism of the cytostatic action of 5-hydroxymethyldeoxyuridine [hmdUrd, a component of ϕe phage DNA] on tissue culture cells, as discovered by Waschke et al. (29). We want to determine whether hmdUrd is phosphorylated (by thymidine kinase?), whether hmdUrd is incorporated into animal cell DNA (competing with dTTP?), and how cells can mutate to hmdUrd-resistance.

State of the Field

In all these experiments, we have added depth and breadth to a field in which little basic information was known (3,4,30). It is not clear yet why some DNAs (and RNAs) in general have unusual nucleosides, whether these unusual nucleosides are essential for viral reproduction, and why thymine evolved (instead of direct use of uracil) for most DNAs. I feel it is important to continue these studies to gain new insight into the structure, biosynthesis, and function of DNA. Others (see 1976 Renewal Application) agree with me, and some talented new people are joining our ranks in uracil-DNA research.

REFERENCES

1. Takahashi, I., and Marmur, J. (1963) Nature 197, 794-795.
2. Price, A. R., and Cook, S. J. (1972) J. Virol. 9, 602-610.
3. O'Donovan, G. A., and Neuhard, J. (1970) Bacteriol. Rev. 34, 278-343.
4. Hemphill, H. E., and Whiteley, H. R. (1975) Bacteriol. Rev. 39, 257-315.
5. Mathews, C. K. (1971) Bacteriophage Bciochemistry, Van Nostrand-Reinhold Co., New York.
6. Roscoe, D. H. (1969) Virology 38, 520-526, 527-537.
7. Kahan, F. (1963) Federation Proc. 22, 406.
8. Price, A. R., and Fogt, S. M. (1973) J. Biol. Chem. 248, 1372-1380.
9. Tomita, F., and Takahashi, I. (1969) Biochim. Biophys. Acta 179, 18-27.
10. Price, A. R. (1974) J. Virol. 14, 1314-1317.
11. Gass, K. B., and Cozzarelli, N. R. (1973) J. Biol. Chem. 248, 7688-7700.
12. Clements, J. E., D'Ambrosio, J., and Brown, N. C. (1975) J. Biol. Chem. 250, 522-526.
13. Price, A. R., and Fogt, S. M. (1973) J. Virol. 11, 338-340.

14. Post, L., and Price, A. R. (1975) J. Virol. 15, 363-371.

15. Price, A. R., and Frabotta, M. (1972) Biochem. Biophys. Res. Commun. 48, 1578-1585.

16. Lavi, U., Nattenberg, A., Ronen, A., and Marcus, M. (1974) J. Virol. 14, 1337-1342.

17. Price, A. R., and Fogt, S. M. (1973) J. Virol. 12, 405-407.

18. Price, A. R., Hitzeman, R., Frato, J., and Lombardi, K. (1974) Nucleic Acids Res. 1, 1497-1502.

19. Price, A. R., and Frato, J. (1975) J. Biol. Chem. 250, 8804-8811.

20. Rima, B. K., and Takahashi, I. (1973) Canad. J. Biochem. 51, 1219-1224.

21. Clark, S., Losick, R., and Pero, J. (1974) Nature 252, 21-24.

22. Dunham, L. T., and Price, A. R. (1974) Biochemistry 13, 2667-2672.

23. Dunham, L. T., and Price, A. R. (1974) J. Virol. 14, 709-712.

24. Friedberg, E. C., Ganesan, A. K., and Minton, K. (1975) J. Virol. 16, 315-321.

25. Tomita, F., and Takahashi, I. (1975) J. Virol. 15, 1073-1080, 1081-1087.

26. Beck, C., Eisenhardt, A. R., and Neuhard, J. (1975) J. Biol. Chem. 250, 609-616.

27. Marcus, M., and Newlon, M. (1971) Virology 44, 83-93.

28. Price, A. R., Dunham, L. F., and Walker, R. L. (1972) J. Virol. 10, 1240-1241.

29. Waschke, S., Reefschläger, J., Bärwolf, D., and Langen, P. (1975) Nature 255, 629-630.

30. Hall, R. H. (1971) The Modified Nucleosides in Nucleic Acids, Columbia Univ. Press, New York.

BIBLIOGRAPHY ON CONTRACT E(11-1)-2101

C00-2101-1
1971 Technical Progress Report
Alan R. Price
To A.E.C. (8 pages).

C00-2101-2
Enzymology of Uracil-DNA Synthesis by PBS2 Phage in Bacillus subtilis.
Alan R. Price
Abstracts, 1971 Bacteriophage Meetings, Cold Spring Harbor, N. Y., p. 81.

C00-2101-3
New Deoxyribonucleic Acid Polymerase Induced by Bacillus subtilis Bacteriophage PBS2.
Alan R. Price and Sandra J. Cook
J. Virol. 9, 602-610 (1972).

C00-2101-4
1972 Technical Progress Report
Alan R. Price
To A.E.C. (7 pages).

C00-2101-5
New Deoxyribonucleic Acid Polymerase Induced by Bacteriophage PBS2 during Infection of Bacillus subtilis.
Alan R. Price
Abstracts, 1972 Ann. Meet. Amer. Soc. Micro., p. 243.

C00-2101-6
Thymidine Triphosphate Nucleotidohydrolase and Deoxyuridylate Hydroxymethylase Induced by Mutants of Bacillus subtilis Bacteriophage SP82G.
Alan R. Price, Linda F. Dunham, and Robert L. Walker
J. Virol. 10, 1240-1241 (1972).

C00-2101-7
Resistance of Bacteriophage PBS2 Infection to Rifampicin, an Inhibitor of Bacillus subtilis RNA Synthesis.
Alan R. Price and Mary Frabotta
Biochem. Biophys. Res. Commun. 48, 1578-1585 (1972).

C00-2101-8
Deoxythymidylate Phosphohydrolase Induced by Bacteriophage PBS2 during Infection of Bacillus subtilis.
Alan R. Price and Suzanne M. Fogt
J. Biol. Chem. 248, 1372-1380 (1973).

C00-2101-9
Infection of Bacillus subtilis by PBS2 Phage Whose DNA Contains Uracil.
Alan R. Price
Abstracts, 1972 Bacteriophage Meetings, Cold Spring Harbor, N. Y., p. 52.

C00-2101-10
Deoxyribonucleotide Metabolism after φe Infection of Bacillus subtilis.
Linda T. Dunham and Alan R. Price
Abstracts, 1972 Bacteriophage Meetings, Cold Spring Harbor, N. Y., p. 51.

C00-2101-11
Resistance of Bacteriophage PBS2 Infection to 6-(p-Hydroxyphenylazo)-Uracil, an Inhibitor of Bacillus subtilis Deoxyribonucleic Acid Synthesis.
Alan R. Price and Suzanne M. Fogt
J. Virol. 11, 338-340 (1973).

C00-2101-12
Effect of Nalidixic Acid on PBS2 Bacteriophage Infection of Bacillus subtilis.
Alan R. Price and Suzanne M. Fogt
J. Virol. 12, 405-407 (1973).

C00-2101-13
1973 Technical Progress Report
Alan R. Price
To A.E.C. (8 pages).

C00-2101-14
1973 Comprehensive Report (first 3 years)
Alan R. Price
To A.E.C. (6 pages).

C00-2101-15
Deoxythymidine Triphosphate-Deoxyuridine Triphosphate Nucleotidohydrolase Induced by Bacillus subtilis Bacteriophage φe.
Linda T. Dunham and Alan R. Price
Biochemistry 13, 2667-2672 (1974).

C00-2101-16
Mutants of Bacillus subtilis Bacteriophage φe Defective in dTTP-dUTP Nucleotidohydrolase.
Linda T. Dunham and Alan R. Price
J. Virol. 14, 709-712 (1974).

C00-2101-17
Properties and Role of the dTTPase-dUTPase Induced by Bacillus subtilis Phage φe.
Linda T. Dunham and A. R. Price
Abstracts, 1973 Bacteriophage Meetings, Cold Spring Harbor, N. Y., p. 23.

C00-2101-18
Bacteriophage PBS2-Induced Deoxycytidine Triphosphate Deaminase in *Bacillus subtilis*.
Alan R. Price
J. Virol. 14, 1314-1317 (1974).

C00-2101-19
Bacillus phage PBS2-Induced dCTP Deaminase and dUTPase Inhibitor.
Alan R. Price
Federation Proc. (Abstracts) 33, 1488 (1974).

C00-2101-20
1974 Technical Progress Report
Alan R. Price
To A.E.C. (6 pages).

C00-2101-21
Rifampicin-Resistant Bacteriophage PBS2 Infection and RNA Polymerase in *Bacillus subtilis*.
Alan R. Price, Ronald Hitzeman, Joan Frato, and Ken Lombardi
Nucleic Acids Res. 1, 1497-1502 (1974).

C00-2101-22
Effects of Inhibitors on Macromolecular Synthesis after PBS2 phage Infection.
Alan R. Price
Abstracts, 1974 Bacteriophage Meetings, Cold Spring Harbor, N. Y., p.96.

C00-2101-23
Inhibition of Bacteriophage PBS2 Replication in *Bacillus subtilis* by Phleomycin.
Leonard Post and Alan R. Price
J. Virol. 15, 363-371 (1975).

C00-2101-24
1975 Technical Progress Report
Alan R. Price
To A.E.C. (7 pages).

C00-2101-25
Bacillus subtilis Deoxyuridinetriphosphatase and Its Bacteriophage PBS2-Induced Inhibitor.
Alan R. Price and Joan Frato
J. Biol. Chem. 250, 8804-8811 (1975).

C00-2101-26
A Bacteriophage-Induced Inhibitor of a Host Enzyme.
Alan R. Price
Microbiology 1976 (ed., D. Schlessinger), in press [presented at the ASM Conference on Bacilli at Cornell University, August, 1975].

STUDENTS TRAINED IN RESEARCH FOR CONTRACT E (11-1)-2101

Graduate students with Dr. Price as mentor:

Linda Frances Thompson Dunham: research on ϕe dTTPase-dUTPase on this contract from June, 1970 to June, 1973, earning Ph.D. in Biological Chemistry.

Ronald Arthur Hitzeman: research on PBS2 DNA polymerase on this contract from June, 1972 to date; expecting to earn Ph.D. in 1976.

Graduate students under Dr. Price for two-month projects:

Richard Ruettinger (1971) on PBS2 DNA polymerase.

Susan Effertz (1971) on ϕe DNA synthesis.

Anne Cahill (1972) on PBS2 resistance to rifampicin.

Mary Frabotta (1972) on PBS2 RNA synthesis.

Evelyn Hansen (1974) on early rifampicin effects on PBS2.

William Mattes (1975) on PBS2 dUMP kinase purification.

Andrew Smith (1975) on PBS2 inhibition of host's N-glycosidase.

Mary Koeppe (1976) on PBS2 DNA synthesis in pol III^{ts} hosts.

Undergraduates under Dr. Price for eight- to twelve-month projects:

Leonard Post (1974-75) on phleomycin effects on PBS2 DNA.

Guy Katz (1975-76) on PBS2 inhibitor of host's N-glycosidase and infected cell pools.

Undergraduates under Dr. Price for four-month projects:

Sandra Cook (1971) on PBS2 DNA polymerase.

Robert Walker (1971) on SP82 hydroxymethylase.

Mariane Wielgosz (1972) on host's dUTPase.

David Reames (1973) on PBS2 DNA polymerase.

Carol Johnson (1974) on PBS2 DNA synthesis at high pH.

Barry Christian (1974) on PBS2 enzyme induction in various media.

Frank Kutyla (1974) on PBS2 DNA polymerase.

Lina Ong (1975) on PBS2 deoxynucleotide pools separation.

Bruce Kolberg (1975) on PBS2 DNA polymerase in polC hosts.

Deborah Kish (1975) host's dUTPase and PBS2 infection at high pH.

Mark Larzalere (1975) PBS2 infection in different media.

Chauncey MacHargue (1976) PBS2 inhibition of host's N-glycosidase.

This contract is my primary (and only federal) support, providing enough funds for supplies plus my technician's salary, part of my salary, and indirect costs.