Amber Mutants of Bacteriophage T4 Defective in Deoxycytidine Diphosphatase and Deoxycytidine Triphosphatase

ON THE ROLE OF 5-HYDROXYMETHYL-CYTOSINE IN BACTERIOPHAGE DEOXYRIBONUCLEIC ACID*

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SUMMARY

Infection of Escherichia coli B with T4 amber mutants in gene 56 fails to cause the appearance of either deoxycytidine triphosphatase or deoxycytidine diphosphatase, and little or no DNA synthesis results. Infection of E. coli B with a 7:3 mixture of a gene 56 amber mutant and wild type T4 results in the appearance of only 30% as much dCTPase activity as normal but gives a 100% yield of mixed phage. This indicates that dCTPase is present in at least a 3-fold excess in the wild type infection.

Escherichia coli W4597, shown by others to support the synthesis by wild type T4 of DNA in which the hydroxymethylcytosine is virtually unglucosylated, also fails to support the synthesis of DNA upon infection with a gene 56 amber mutant. This and other evidence indicate that in T4 DNA a crucial function of hydroxymethylcytosine, whether glucosylated or not, is the protection of progeny phage DNA against nuclease activity that degrades the cytosine-containing DNA of the host.

The enzyme deoxycytidine triphosphatase (EC 3.6.1.12, dCTP nucleotidohydrolase) was discovered by Koerner, Smith, and Buchanan (1) and independently by Kornberg et al. (2) in extracts of Escherichia coli infected with bacteriophage T2. Zimmerman and Kornberg offered strong evidence that the enzyme can dephosphorylate not only dCTP but also deoxycytidine diphosphate; deoxycytidine monophosphate is formed in both cases (3). In fact, Warner and Barnes (4) recently showed that the phage-directed deoxuridine triphosphatase and deoxuridine diphosphatase activities discovered by Greenberg (5) are also due to the dCTPase. At least one function of this enzyme has appeared obvious, in that it can account for the lack of cytosine in T-even phage DNA (1, 2). Why these phages substitute 5-hydroxymethylcytosine for cytosine has been less obvious. The present studies provide an answer.

In order to be able to investigate the intracellular functions of dCTPase, phage mutants unable to produce it were sought. Earlier attempts by others* and by the author† to isolate dCTPase mutants as pseudorevertants‡ of dCMP hydroxymethylase amber mutants (6, 7) were unsuccessful. The working hypothesis was that such "revertants" would make progeny, the DNA of which contained cytosine instead of HMC. Such DNA would be unglucosylated and therefore it was expected that progeny containing such DNA would have the properties of T* phage (8-12). The latter replicate on certain Shigella strains but not on E. coli B. A similar scheme was used successfully by Revel, Hattman, and Luria to isolate T2 mutants defective in α-glucosyltransferase. Such mutants grow on Shigella but not on E. coli B (13). The failure of this scheme to produce dCTPase mutants is now understandable in that such mutants do not direct the synthesis of phage DNA, for reasons discussed below.

At this point a number of newer amber mutants from Edgar were examined, and it was found that those shown by Edgar* to map in gene 56, and also to direct the formation of little or no DNA, were dCTPase-. This observation, which was reported briefly earlier (14), is documented in the present publication. It is shown that (a) the mutants also fail to direct the

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† J. Flatgaard, personal communication.

‡ S. Hattman, personal communication.

‡ J. S. Wiberg, unpublished experiments.

* These would be double mutants defective in both dCMP hydroxymethylase and dCTPase.

† The abbreviations used are: HMC, 5-hydroxymethylcytosine; dHMP, deoxy-5-hydroxymethylcytidine 5'-monophosphate; T*, T-even phage, the DNA of which lacks the glucose normally attached to HMC groups; am, amber mutants, unable to grow on E. coli B but able to grow on E. coli CR63; ts, temperature-sensitive mutants.

‡ R. S. Edgar, personal communication.
Materials and Methods

Escherichia coli Strains—W3110 is a gal derivative of W3110 (15) and produces T*4 phage. Both of these are K-12 strains and originated in the laboratory of Drs. F. M. and J. Lederberg. They were kindly supplied by Dr. T. Fukasawa and are restrictive for all of the amber mutants used in the infections for Fig. 2. K803 and B834 were the generous gift of Drs. W. B. Wood (16); K803 has lost the ability to restrict λ-B, i.e. bacteriophage λ grown on E. coli B, and is also permissive for T*4 and for all the T4 am mutants used in the infections for Fig. 2. B834 has lost the ability to restrict K-λ, i.e. bacteriophage λ grown on E. coli K12, but is restrictive for T*4 and for all of the T4 am mutants used in the infections for Fig. 2. Sources of other strains are given in the text or were described previously (14).

Phage Strains—Phage T4w is the wild type T4D. All am and ts mutants were the generous gift of Dr. R. Edgar. The suffix "x5" indicates that such mutants were purified genetically in this laboratory by crossing five times against T4w, at a ratio of 10 T4w to 1 am. That such purification is eminently justified is clear from our demonstration that amC153 and amB14 each contains a ts mutation (in an unknown gene) in addition to the am lesion. These ts lesions are not present in amC153x1 and amB14x5. Mutant amA56x5 has retained the ts property of amA56; thus, this property is probably inherent in an altered gene 47 product produced in the permissive strain CR83. In support of this is the fact that an independently isolated mutant mapping at the same site, namely amA516 (from A. Bolle), is also ts on CR83. The construction of multiple mutants and the preparation of phage stocks have been described (14).

Growth Media—Bacteria were grown, and infections carried out, in the glycerol-Casamino acids medium of Fraser and Jer银川 (17). Assay procedures for viable phage and bacteria were described previously (14).

Chemicals—2-14C-dTMP was purchased from Schwarz Bio-Research, Inc. Dreft is a detergent produced by Procter and Gamble and contains 15% sodium dodecyl benzene sulfonate. The dUTPase assay used previously (14) was modified by adding EDTA to the reaction mixture to a final concentration of 2.5 × 10^{-4} M. This modification was suggested by Dr. H. R. Warner. It increases the rate by 30 to 40% and increases the reproducibility. The dCDPase assay was identical with that for dCTPase, except that dCDP was substituted for dCTP. The incubation for the dUTPase assay was identical with that for dCTPase, except that dUTP was substituted for dCTP. The isolation of DUMP from the reaction mixture was accomplished with a modification of the procedure of Warner and Barnes (4), as follows. After addition of 10 μmoles of EDTA to stop the reaction, the mixture was applied to a column (30 × 3.25 mm) of Dowex 1-10X (formate), 200 to 400 mesh. The reaction tube was rinsed with 2 ml of water, and this was used to wash the column. The column was next washed with 2 ml of 0.05 M ammonium formate buffer, pH 4.35. Three milliliters of 0.25 M ammonium formate buffer, pH 4.35, were then applied to remove DUMP. This was collected and its absorbance at 202 μg was determined.

For the dTMP kinase assay, the reaction mixture was identical with that previously described for dHMP kinase (7), except that dTMP was substituted for dHMP. 2-14C-dTMP amounting to about 1000 cpm of radioactivity and extract equivalent to about 5 × 10^{4} cells were used per assay vessel. Reagent and extract were incubated for 40 min at 37°; then 0.4 ml of 0.025 M EDTA, pH 7 to 8, was added to stop the reaction. The mixture was applied to a column (30 × 3.25 mm) of Dowex 1-10X (formate), 200 to 400 mesh. The tube was rinsed with 1 ml of water, and this was used to wash the column; then 10 ml of 0.25 M ammonium formate buffer, pH 4.35, were washed through the column. The DTMP and dUTP were then eluted with 2 ml of 2 M ammonium formate buffer, pH 4.35, and collected in a ventilation hood on a stainless steel planchet placed on a hot plate, the surface temperature of which was 95–100°. We used a Pandus surface thermometer (Pacific Transducer Corporation, Los Angeles, California), the accuracy of which was confirmed with boiling water. A nonwettable ring was drawn at the periphery of the planchets (Footnote 17 in Reference 22). To keep the 2 M buffer from running to the edge of the planchet, H. R. Warner, personal communication.

Enzyme Assays—In each of the following assays the activities reported for extracts of infected cultures were calculated from the raw data after the values observed for the extract of uninfected cells had been subtracted. The upper limits of activities in uninfected cells are indicated in the text. Enzyme activity is expressed on a per cell basis, rather than per milligram of protein, in order to indicate directly the absolute amounts of enzyme. This is particularly important when mutant phage are compared with wild, since it has been shown that protein synthesis continues more or less unabated after infection by wild type T-even phage (19, 20), and it is to be expected that the extent of this protein synthesis will vary from mutant to mutant, depending on the gene defect. Nonetheless, to facilitate comparison with other literature values for these enzyme activities, the total protein content of the uninfected bacteria grown under the same conditions used here was determined. The values were 354 mg per 10^{12} cells for E. coli B, and 200 mg per 10^{12} cells for E. coli W3110. Cell count was based on colony formers (14). Protein content was determined by precipitating the cells in cold 10% trichloracetic acid, sedimenting at 5000 × g, dissolving the pellet in 0.1 M NaOH at 25° for at least 1 hour, and applying to this solution the assay of Lowry et al. (21). The standard was crystalline bovine albumin (Nutritional Biochemicals).

The dCTPase assay used previously (14) was modified by adding EDTA to the reaction mixture to a final concentration of 2.5 × 10^{-4} M. This modification was suggested by Dr. H. R. Warner. It increases the rate by 30 to 40% and increases the reproducibility. The dCDPase assay was identical with that for dCTPase, except that dCDP was substituted for dCTP. The incubation for the dUTPase assay was identical with that for dCTPase, except that dUTP was substituted for dCTP. The isolation of DUMP from the reaction mixture was accomplished with a modification of the procedure of Warner and Barnes (4), as follows. After addition of 10 μmoles of EDTA to stop the reaction, the mixture was applied to a column (30 × 3.25 mm) of Dowex 1-10X (formate), 200 to 400 mesh. The reaction tube was rinsed with 2 ml of water, and this was used to wash the column. The column was next washed with 2 ml of 0.05 M ammonium formate buffer, pH 4.35. Three milliliters of 0.25 M ammonium formate buffer, pH 4.35, were then applied to remove DUMP. This was collected and its absorbance at 202 μg was determined.
from the chilled cultures by sonic treatment (14). The residue on the plate was then spread evenly by adding about 10 drops of 0.015% Dreft in 50% ethanol and allowing this to dry on the hot plate. Where liquid scintillation equipment is available for assay of radioactivity, this evaporation step might conveniently be bypassed.

Preparation of Extracts—Extracts were prepared directly from the chilled cultures by sonic treatment (14).

Surviving Bacteria.—After phage infection, surviving bacteria were assayed by spreading 0.1 ml of an appropriate dilution on a glycerol-Casamino acid-agar plate along with 0.1 ml of a suspension of heat-killed bacteria. The latter serve as a practical substitute for antiphage serum by adsorbing any phage which are upper limits and which have been subtracted from the values presented, are dCTPase, 8; dCDPase, 9; and dUTPase, 12.

Excess dCTPase Is Normally Made.—In the attempt to assess the functions of an enzyme in vivo it is useful to ask if its level is quantitatively limiting in any way. Therefore, a mixed infection of E. coli B by both T4 and any am mutant in gene 56 was performed, and dCTPase levels (Fig. 1) and phage production (Table II) were measured. It is seen that although the dCTPase levels in the mixed infection are only 30% of normal, and thus proportional to the input T4 fraction, a full yield of phage is produced. This indicates quite clearly that dCTPase normally is made in at least a 3-fold excess.

### Table I

<table>
<thead>
<tr>
<th>Gene</th>
<th>Activity (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>124</td>
</tr>
<tr>
<td>amN55x5 (gene 42)</td>
<td>261</td>
</tr>
<tr>
<td>amE51x5 (gene 56)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>amC153x1 (gene 56)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>amE114 (gene 56)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>amE56 (gene 56)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>T4 + uninfected</td>
<td>93</td>
</tr>
<tr>
<td>T4 + amE51x5</td>
<td>94</td>
</tr>
<tr>
<td>T4 + amC153x1</td>
<td>96</td>
</tr>
<tr>
<td>T4 + amE114</td>
<td>93</td>
</tr>
<tr>
<td>T4 + amE56</td>
<td>94</td>
</tr>
</tbody>
</table>

* Activities expressed as micromoles of nucleoside monophosphate formed in 60 min in the standard assay (Materials and Methods). In extracts of uninfected cells, the activities, which are upper limits and which have been subtracted from the values presented, are dCTPase, 8; dCDPase, 9; and dUTPase, 12.

The decrease in activity of about 20%, caused by adding the uninfected E. coli B extract to the T4 extract, results from a small drop in pH caused by the phosphate in the growth medium. This effect is not seen when the total volume of extract is less than 50 µl.

It was applied to the column in two portions of 1 ml each. The water from the first addition was allowed to evaporate before the second was added. After the water had evaporated, the ammonium formate took about an hour more to disappear. The ammonium formate was added. After the water had evaporated, the ammonium formate took about an hour more to disappear. Where liquid scintillation equipment is available for assay of radioactivity, this evaporation step might conveniently be bypassed.

### RESULTS

#### Gene 56 Mutants Are Defective in dCTPase and dCDPase

In Table I it is shown that infection of E. coli B by four independently isolated amber mutants in gene 56 results in the appearance of no detectable dCTPase activity. It is seen that dCDPase activity is also missing; thus, genetic support is provided for the biochemical arguments of Zimmerman and Kornberg that the two activities reside in the same protein (3).

### Enzyme and Gene Specificity of Defect

A mutant in gene 42, a structural gene for dCMP hydroxymethylase (22, 24), was also included in the experiment of Table I as a control to illustrate both the specificity of the dCTPase am lesion and the overproduction, or "extended synthesis," of early enzymes seen with mutants that fail to make any DNA (7). Of all the mutants examined by us in other genes, none failed to direct the synthesis of dCTPase. These included all those listed in Table I of Warner and Barnes (4) as directing the synthesis of dUTPase, plus amN134 (gene 33) and 5826 T4 Mutants Defective in dCTPase Vol. 242, No. 24

The same extracts used for the data of Table I were assayed for dTMP kinase activity. The values observed for A through F and uninfected bacteria, respectively, were 16, 20, 15, 15, 17, and less than 0.5 μmole of dTMP phosphorylated per min per 10^10 cells. Thus, the mutations have not affected dTMP kinase. Warner and Barnes have confirmed the latter observation and have shown that mutants in gene 56 also direct the synthesis of dCMP deaminase and dihydrofolate reductase (4).

#### Excess dCTPase Is Normally Made

In the attempt to assess the functions of an enzyme in vivo it is useful to ask if its level is quantitatively limiting in any way. Therefore, a mixed infection of E. coli B by both T4 and an am mutant in gene 56 was performed, and dCTPase levels (Fig. 1) and phage production (Table II) were measured. It is seen that although the dCTPase levels in the mixed infection are only 30% of normal, and thus proportional to the input T4 fraction, a full yield of phage is produced. This indicates quite clearly that dCTPase normally is made in at least a 3-fold excess.

We have found that the genetic map positions of mutants amE51x5 and amE56 are identical or very close together, and that the map positions of amC153x1 and amE114 are identical or very close together, but at a different site, separable from the first with a recombination frequency of about 4% in the permissive host CR63. A recent genetic map of phage T4, including gene 56, has been published by Edgar and Wood (23).
Evidence That Cytosine, Not Lack of Glucose, Confers DNase Lability on T4 DNA—It was shown previously (14) that infection of E. coli B by a dCTPase mutant results in the synthesis of very little DNA, and that even this is later degraded. It was also shown that, by blocking genetically DNase activity that normally degrades the host DNA, dCTPase mutants can direct the synthesis of much DNA that remains acid-insoluble. It was concluded that the main reason for the lack of accumulation of DNA upon infection by a dCTPase mutant was that cytosine was replacing HMC in the DNA and thus rendering the DNA susceptible to DNase action. This result prompts the question of whether the lability of the cytosine-containing progeny DNA is due to the presence of cytosine, per se, or to the fact that the cytosine is not glucosylated. Certain strains of E. coli that are defective in the synthesis of UDP-glucose, the precursor (2) of the glucose attached to HMC, have been shown to support the synthesis of functional T-even phage DNA in which the HMC groups are essentially unglucosylated (9-12).

Thus, in such strains, glucosylation of the progeny DNA does not appear to be necessary for maintaining its integrity during its synthesis; but since the intracellular DNases could differ between these strains and E. coli B, we cannot logically extrapolate to E. coli B this argument regarding glucosylation.

Consequently, the experiment represented in Fig. 2 was performed. It is seen that in such a UDP-glucose-deficient strain (W4597), just as in E. coli B, little or no DNA is synthesized when the infecting phage is a mutant defective in dCTPase (amE51x5). It also can be seen in Fig. 2 that by blocking genes 46 and 47, shown previously to control DNase activity responsible for degradation of the bacterial DNA (14), DNA does accumulate despite the block in gene 56. In fact, as is also shown in Fig. 2, infection by a mutant in which genes 42, 46, 47, and 56 are blocked still results in as much DNA synthesis as occurs when only genes 46 and 47 are blocked. This DNA must contain cytosine to the absolute exclusion of HMC, since the block in gene 42 prevents formation of dHMP, the HMC-containing nucleotide. These results argue that the failure of DNA synthesis with the gene 56 am mutant is due primarily to DNase action and not to some other possible consequence of the dCTPase lesion. The main conclusion from this experiment is that progeny 14 DNA containing unglucosylated HMC.

### Table II

<table>
<thead>
<tr>
<th>Phage</th>
<th>Adsorbed multiplicity of infection (2 min)</th>
<th>Phage production per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adsorbed multiplicity of infection (2 min)</td>
<td>30 min</td>
</tr>
<tr>
<td>A. T4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.3</td>
<td>7.1</td>
</tr>
<tr>
<td>B. amE51x5</td>
<td>6.9</td>
<td>6.0</td>
</tr>
<tr>
<td>C. T4&lt;sub&gt;+&lt;/sub&gt; + amE51x5</td>
<td>8.5</td>
<td>7.5</td>
</tr>
<tr>
<td>T4&lt;sub&gt;+&lt;/sub&gt; + amE51x5</td>
<td>8.3</td>
<td>6.0</td>
</tr>
</tbody>
</table>

At 2 min, 0.1 ml was removed into broth saturated with chloroform and assayed later for unadsorbed phage; this value was subtracted from the input multiplicity of infection to give the value in the column labeled "Adsorbed Multiplicity of Infection." Any phage adsorbing after this time can be ignored because of genetic exclusion (25).

Since in the mixed infection (Line C) the multiplicity of infection for T4<sub>+</sub> is 2.2, only 80% of the cells are infected with one or more T4<sub>+</sub> (36); thus, only this fraction of cells can be expected to yield phage. The values in parentheses (Line A) represent 80% of the T4<sub>+</sub> phage production values, and it is these figures that should be compared with those in Line C.  

* Equal to 30% of total.  
* Equal to 28% of total.  
* Equal to 31% of total.
Fig. 2. DNA production after infection of E. coli W4597, a strain unable to glucosylate T4 phage DNA. Numbers in brackets identify the genes mutated: A, ○, T4; B, ■, amN55x5 [42]; C, ○, amE51x5 [56]; D, △, amB14x5, amA456x5 [40, 47]; E, △, amB14x5, amA456x5, amE51x5 [46, 47, 56]; F, *, amN55x5, amB14x5, amA456x5, amE51x5 [42, 46, 47, 56]; +, uninfected bacteria. An overnight culture of E. coli W4597 was diluted 1:100 into fresh glycerol-Casamino acid medium and aerated at 37° for approximately 3 hours, when a concentration of 5.2 x 10⁸ viable cells per ml was reached. The culture was chilled until used (within 2 hours). For each infection 20 ml were prewarmed for 3 min by shaking at 37° in a notched, 250-ml Erlenmeyer flask. At zero time and again at 3 min, 0.1 ml of phage suspension was added to give an input multiplicity of infection of 5 each time. At the times indicated, aliquots of 2 ml were taken for DNA assay. At 6 min, 0.1 ml was diluted into 10 ml of cold glycerol-Casamino acid medium and later assayed for surviving bacteria; in all cases less than 5% survived, indicating an effective multiplicity of infection of over 3. An aliquot of 2 ml was chilled at 20 min for preparation of enzyme extract. At 50 min, 0.1 ml was diluted into 10 ml of broth saturated with chloroform and assayed for phage. The results, expressed as phage per cell, were as follows: A, 111 on E. coli K903, and 0.6 on E. coli B834; B through F, all less than 1 on E. coli K803. All of these values less than 1 probably represent unadsorbed input phage.

is not degraded, whereas that containing a high proportion of cytosine is degraded. The most likely reason for this is that one or more of the nucleases concerned is specific for cytosine DNA and does not attack HMC-DNA.

To test the general success of the infection, the activity of dTMP kinase was measured in extracts taken at 20 min. The values were 13, 12, 14, 17, 15, 12, and less than 0.5 amole per min per 10⁸ cells for A through F and uninfected bacteria, respectively, an indication of successful infection in all cases. That W4597 did indeed produce glucose-deficient phage (T₄*) was evidenced by the fact that the progeny of the infection by T₄w (Fig. 2) grew on K803 (a strain permissive for T₄*) but not on B834 (a strain restrictive for T₄*). Normally glucosylated T₄w was shown to grow equally well on both strains.

An unexpected result was that in W4597, the double mutant blocked in genes 46 and 47 produced a negligible yield of phage (Fig. 2), i.e. less than 10% of the 5 to 10 phages per cell seen on E. coli B (6, 14) or on W3110, the glucosylating parent of W4597. The reason for this is obscure, but, since maturation of a gene 46 or 47 mutant is so poor even in E. coli B, perhaps it can be viewed as an exaggeration of the 5- to 10-min delay in phage production in W4597 relative to that in W3110, which we have observed even with T₄w (see "Discussion").

**DISCUSSION**

The present results are consistent with the conclusion that gene 56 is a structural gene for dCTPase; an alternative, for which there is little precedent in phage systems, is that it is a regulator gene. It is expected that studies in progress on dCTPases directed by ts mutants, or am mutants in permissive hosts, will clarify this question.

The finding that dCTPase is normally made in excess agrees with our similar observations on two other T₄ early enzymes, dCMP-hydroxymethylase and diHMP-5MP-5MP kinase. ³ Other kinds of evidence have led to the same conclusion for two other early functions, rII (27-29) and thymidylate synthetase (30). Eiserling and Epstein have measured the decrease in burst size as a function of the proportion of wild type T₄ in mixed infections with am mutants defective in a variety of genes. ¹⁰ They found that several proteins related to head production were limiting, but that there was a tendency for enzymes or minor structural proteins to be made in excess.

Although the simplest hypothesis to explain the phenotype of mutants in genes 46 and 47 is that these genes encode the structure of a DNase, other explanations are possible. For example, the products of genes 46 and 47 may in some way activate, or divert to the purposes of the phage, a bacterial DNase. If so, a bacterial strain lacking this enzyme might be expected to mimic the effect of a gene 46 or 47 defect by allowing the accumulation of cytosine-containing T₄ DNA upon infection by a dCTPase mutant. We have examined four bacterial strains suspected or known to have DNase defects, and have found that dCTPase mutants failed to cause the synthesis of significant amounts of DNA in all four. These were (a) *Shigella dysenteriae* strain Sh 16 (from S. Hattman), which is permissive for T₄ phage (8, 9); (b) E. coli B834 (from W. Wood), which is permissive for bacteriophage λ grown on a K12 strain (16); (c) E. coli W3102 rec (from M. Meselson), which is defective in genetic recombination and is also sensitive to ultraviolet irradiation; and (d) E. coli K12-1100 (isolated by H. Hoffman-Berling and obtained from G. R. Greenberg), which is defective in endonuclease I. It is concluded that if these bacterial strains are indeed functionally defective in one DNase or another, those DNases are not involved in any obligatory way in the degradation of cytosine-containing T₄ DNA observed upon infection by a dCTPase mutant.

Our argument, that glucose need not be attached to HMC in progeny phage DNA in order that this HMC protect the DNA from nucleases, could be objected to in that Fukasawa and Sato (31) showed that the glucose content of T₄ made on W4597 was, in fact, about 10% of normal. Thus, it is conceivable that a level of glucosylation somewhere between 0 and 10% of normal is required to protect progeny DNA from nucleases.

10 F. Eiserling, personal communication.
11 M. Meselson, personal communication.
and that substitution of cytosine for HMC necessarily decreases the extent of glucosylation below this critical level. Evidence against this objection resides in the fact that T*2 and T*6 made on W4597 contain demonstrably less glucose (12, 31), and in the demonstration by Davison and Freifelder (32) that the DNAs of T*2 and T*6 made on E. coli B/40 contain less than 1% of the normal amount of glucose. A strict test of our hypothesis must await purification of the appropriate DNase, or DNases, and studies of its base specificity.

Warner and Barnes (4) have pointed out that since gene 56 am mutants are also defective in dUTPase, any DNA accumulating in infections by such mutants may contain uracil as well as cytosine, and that any unusual properties of such DNA may be attributable to either base or to both. In the one case so far examined, Kutter and Wiberg (33) found no detectable uracil (i.e. less than 2% substitution for thymine) in the DNA of viable T4 phage produced in an infection of E. coli B by a temperature-sensitive dCTPase mutant at a partially restrictive temperature. In this same DNA, cytosine replaced 20% of the HMC normally present. Since purified DNA-nucleotidyltransferase of T4 can accept dUTP as a substrate (34) it seems probable that the bacterial dUTPase (33, 34) is adequate to keep uracil out of phage DNA despite the loss of the phage-transferase of T4 can accept dUTP as a substrate,13 it seems probable that the bacterial dUTPase (33, 34) is adequate to keep uracil out of phage DNA despite the loss of the phage-transferase of T4.

In regard to our observation of a delay in T4 production in W4597 relative to W3110, it should be noted that Fukasawa and Saito (31) observed no difference between the two strains in W4597 relative to W3110, to support phage production is temperature-sensitive dCTPase mutant at a partially restrictive temperature. In this same DNA, cytosine replaced 20% of the HMC normally present. Since purified DNA-nucleotidyltransferase of T4 can accept dUTP as a substrate,13 it seems probable that the bacterial dUTPase (33, 34) is adequate to keep uracil out of phage DNA despite the loss of the phage-transferase of T4.

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Amber Mutants of Bacteriophage T4 Defective in Deoxycytidine Diphosphatase and Deoxycytidine Triphosphatase: ON THE ROLE OF 5-HYDROXYMETHYLICYTOSINE IN BACTERIOPHAGE DEOXYRIBONUCLEIC ACID

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