Role of Deoxycytidylylate Deaminase in Deoxyribonucleotide Synthesis in Bacteriophage T4 DNA Replication*

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During the infection by bacteriophage T4, dTMP and 5-hydroxymethyl dCMP (HmdCMP) are synthesized in the ratio of 2:1, respectively, i.e. at the Thy/HmCyt ratio found in T4 DNA. This study demonstrates that on infection by a cd (phage-induced dCMP deaminase) mutant the dTMP/HmdCMP synthesis ratio is approximately 0.6. The ratio remains about 0.6 on infection by cd dna double mutants. At the same time, with cd the combined rate of dTMP plus HmdCMP synthesis increases by about 50% over the wild type rate. The rates of synthesis of dTMP and of DNA with phage cd appear to remain the same as with phage T4. However, free HmdCMP derivatives are greatly overproduced and accumulate. From these experiments, it has been estimated that in wild type phage infections approximately 40% of the dTMP derives from dCMP.

Treatment of a T4D-infected culture by proflavine 10 min after infection blocks the synthesis of both dTMP and HmdCMP, but eventually the ratio of dTMP/HmdCMP synthesis increases above 2:1. With cultures infected by cd, the synthesis of dTMP and HmdCMP is also inhibited, but the ratio does not increase above 0.6:1. Thus the rise in ratio which proflavine causes in wild type phage infection requires the dCMP deaminase pathway.

These experiments demonstrate that dCMP deaminase plays an important quantitative role in maintaining the correct ratio of dTMP/HmdCMP synthesis. Regulation of the ratio at 2:1 depends on factors intrinsic to the complex of enzymes synthesizing deoxyribonucleotides (Flanagan, J. B., and Greenberg, G. R. (1977) J. Biol. Chem. 252, 3019–3027). These deoxyribonucleotides are synthesized at a constant ratio of 2.1 to 1, respectively, exactly reflecting the ratio of thymine to 5-hydroxymethylcytosine in T4 DNA. This ratio of synthesis is maintained even on infection by T4 DNA- mutants until the deoxyribonucleotides reach very high concentrations (2). As a result, it has been proposed that the primary control is not through effector-sensitive enzymes, but is the inherent property of a complex of enzymes synthesizing deoxyribonucleotides (2, 3) and channeling the products into the DNA growing points, i.e. deoxyribonucleotide synthetase (4). Agents altering DNA inhibit the synthesis of dTMP and HmdCMP and alter their ratio of synthesis. Accordingly, we have suggested that template DNA has a structural/ regulatory role in the deoxyribonucleoside synthetase complex (5).

T4 DNA polymerase appears to be necessary for the maximum synthesis of pyrimidine deoxyribonucleotides (3). At the same time, dCMP hydroxymethylase and HmdCMP kinase have been shown to have indispensable second roles in the replication process (6–10). Thus the deoxyribonucleotide enzymes are regulated through DNA and form a mutually dependent system with the replication apparatus.

dCMP deaminase activity induced by phage T2 infection was first reported in 1959 by Keck et al. (11). Hall and Tessman in 1966 described phage T4 mutants (cd) unable to induce the formation of dCMP deaminase and considered that the enzyme could have an accessory role in the synthesis of dTMP (12). The dCMP deaminases occurring after infection by phages T4 and T6 and with phage T2 have been studied extensively and shown to be effector-sensitive by Bessman and co-workers (13, 14) and Maley et al. (15), respectively. Our experiments with T4 DNA- mutants appear to rule against a feedback-sensitive regulatory role for the enzyme in the in vivo control of the synthesis of dTMP and HmdCMP (2). However, the present studies show that this enzyme has an important quantitative role in augmenting the synthesis of dTMP and therefore in maintaining the 2:1 Thy/HmCyt ratio.

EXPERIMENTAL PROCEDURES

Biological Materials—The bacterium employed in all the kinetic studies was a B strain, Escherichia coli thyA deoB (GM 201), described previously (16). Amber strains of T4 phage were grown on E. coli CR63 (Su14). Bacteriophage T4D and the amber mutants were employed earlier (1, 2). T4 phage cdN16, mutant in the gene

Bacteriophage T4-infected cultures possess a remarkable system controlling the synthesis of dTMP and HmdCMP (1–3). This work was supported by United States Public Health Service Grant AI01973 from the National Institutes of Health and Grant NP-138A from the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: HmdCMP, 5-hydroxymethyl dCMP; HmCyt, 5-hydroxymethylcytosine; Dna-, Dna-synthesis negative (2).
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determined dCMP deaminase, was obtained from D. H. Hall, Georgia Institute of Technology, Atlanta. Phage were propagated as described (2). Double mutants of cd dna were constructed by standard phage crosses in E. coli C606 (1). The cd mutation was detected by the lack of dCMP deaminase activity in infected extracts, and the dna mutation was confirmed by complementation tests.

Conditions of Infection—The conditions of infection have been described. The multiplicity of infection was 8 and the temperature was 30° (1). Infection was monitored by measurement of dTMP synthetase, dCMP hydroxymethylase, and T4 DNA polymerase and slight variations in the degree of infection corrected as described earlier (1).

Measurement of Tritium Release, Thy/HmCyt Ratios, and DNA Synthesis—The release of tritium from 5-labeled pyrimidine precursors, the ratio of Thy/HmCyt deoxyribonucleotides synthesized via the reductive pathway and DNA synthesis were measured as previously described (1, 5). The release of tritium from administered [5-3H]uridine occurs at the levels of dTMP synthetase and of dCMP hydroxymethylase (Fig. 1) and measures all labeled dTMP and HmdCMP derivatives formed via the reductive pathway including those derivatives incorporated into the DNA and also excreted into the medium (1, 2). The Thy/HmCyt ratio again includes all thymine and HmCyt derivatives synthesized via the reductive pathway from administered [6-3H]uracil. After formic acid hydrolysis of the infected culture, the resulting free thymine and 5-hydroxymethylcytosine bases are separated by thin layer chromatography and their radioactivities measured (2). From the tritium release values and the corresponding Thy/HmCyt ratios the quantities of dTMP and HmdCMP synthesized can be determined. [5-3H]uridine or [6-3H]uracil was at a concentration which inhibits the de novo synthesis of pyrimidine ribonucleotides by feedback (1, 17) (see comment under "Discussion"). DNA synthesis was followed by the incorporation of [5-3H]uridine into the acid-precipitated, alkali-labile fraction analyzed as described (7). Labeled uridine and uracil give equivalent values for tritium release and DNA synthesis (see Footnote 2, Ref. 2).

Enzyme Assays—dTMP synthetase and dCMP hydroxymethylase were assayed as described earlier (1). dCMP deaminase was measured by a modification of the procedure of Scocca et al. (14) using [5-3H]dCMP as the substrate. The resulting labeled dUMP was separated on Dowex 50-H+ columns (14). T4 DNA polymerase was assayed by a modification of the method reported by Goulain et al. (18).

RESULTS

Effect of T4 cd Mutation on Rate of Synthesis of dTMP plus HmdCMP Derivatives—Fig. 1 shows a modified scheme illustrating the pathways of conversion of labeled uracil compounds at the ribonucleotide level through the de novo reductive channel to dTMP and HmdCMP derivatives in phage T4-infected cultures (1, 2, 4). The numbers adjacent to the arrows will be considered under "Discussion." Administered [5-3H]uridine or [6-3H]uracil on conversion through dTMP and HmdCMP releases its tritium atom into the aqueous medium.

In Fig. 2, [5-3H]uridine, to measure the release of tritium, and [6-3H]uracil, to measure DNA synthesis, were administered simultaneously to two cultures infected by T4D and cdN16. Cultures infected by cd phage reached about a 50% increase in the release of tritium from [5-3H]uridine over that shown by T4D infection. It was anticipated that possibly less dTMP synthesis would occur because of the earlier impact of Hall and Tessman (11) that cd mutants gave a reduced phage burst. However, increased tritium release was not consistent with decreased dTMP synthesis, and from the experiments in the following sections, indeed dTMP synthesis did not appear to be decreased.

Fig. 2 (inset) shows that the relative rates of incorporation of [6-3H]uracil into the acid-insoluble, alkali-stable fraction were similar to those in the host DNA breakdown (1-6).

Also called the "de novo" and "reductive de novo" pathway to deoxyribonucleotides, to distinguish it from the system forming deoxyribonucleotides from host DNA breakdown (1-6).

Fig. 1. Synthesis of pyrimidine deoxyribonucleotides in bacteriophage T4 infection. The figure shows the sites of release of H into the aqueous phase from administered [5-3H]uridine. The numbers above and to the left of the arrows represent calculated rates of the nucleotide pathways in T4D infection; those in parentheses are the rates after phage cd infection. The units are nanomoles of labeled compound converted per min per 10^6 infected cells at 30° during the period of linear synthesis (see text).

(dNA) after T4D and cdN16 infection appeared to be equal. However in these experiments, while infections were equivalent by enzyme assays (see legend to Fig. 2), we did not consider variations of less than ±10% as significant. In T4D infection, DNA synthesis was similar to the release of 3H. It is known, in fact, from our previous studies that DNA synthesis and tritium release are exactly equal on infection by T4D. On infection by DNA mutants, tritium release has been shown to be unchanged, i.e. it continued at a rate equal to that of the wild-type phage (1). However, infection by cd phage gives rise to a much greater tritium release than DNA synthesis. Thus, deoxyribonucleotides must accumulate during infection by cd phage.

The ratio of dTMP/HmdCMP synthesis at various times after infection was found to be far lower than the 2-1 ratio found on wild type phage infection. Table I shows that the Thy/HmCyt ratio after infection by cdN16 was about 0.5. The ratio was sometimes found to average as high as 0.78 (see Table III). As reported earlier (2), small quantities of HmCyt derivatives are converted to HmUra derivatives in the infections with DNA mutants. About 3% of HmCyt was found as HmUra in the infections with cdN16. This amount was not included in the calculation of the Thy/HmCyt ratios.

Table II also shows that a double mutant carrying lesions in both the cd gene and the dna gene 44 (cdN16 amN82) or gene 45 (cdN16 amE10) still forms dTMP and HmdCMP in the ratio of about 0.51. We had previously reported that DNA mutants show Thy/HmCyt ratios of 2:1 until 25 min after infection at 30°; then the ratio begins to rise, reaching values of about 4 by 45 min. For comparison, the results of infection by amN82 and by amE10 are shown in the table. Averaging 28 separate ratios in infections by cd and cd dna mutants, ranging from 0.40 to 0.83, gave a value of 0.58:1.

Table II compares the rate of synthesis of DNA with the rates of synthesis of dTMP and HmdCMP derivatives on infection by cd phage. The table also shows the calculated utilization of dTMP and HmdCMP derivatives in the synthesis of DNA. Firstly, the total dTMP + HmdCMP synthesized is about 1.6 times greater than the rate at synthesis of DNA. Based on the fact that thymine constitutes 69% of the pyrimidines in T4 DNA, the rates of utilization of thymine and HmCyt derivatives for DNA synthesis can be calculated (Columns 2, 4, and 6, Table II). The thymine derivatives are utilized at the rate they are formed, in the period between 15 and 45 min. On the other hand, HmCyt derivatives are
accumulated decreases greatly with time. Therefore, the ratio of Thym/HmCyt deoxyribonucleotides formed in great excess of their requirement for DNA synthesis is expressed as pyrimidine nucleotide equivalents incorporated. In extracts obtained at 11 and 13 min from the T4D- and cd-infected cultures, the ratio of dCMP hydroxymethylase and dTMP synthetase activities in the two infections averaged, respectively, 0.92 and 1.05.

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Since DNA is being synthesized during infection by cd phage, a large part of the labeled pyrimidine will be found in the DNA fraction. In our standard procedure for determining the ratio of Thym/HmCyt nucleotides synthesized, the total thymine and HmCyt is measured including that in DNA, and the DNA fraction. In our standard procedure for determining the ratio of Thym/HmCyt deoxyribonucleotides is masked. Therefore, to verify the low Thy/HmCyt ratios calculated for the accumulated deoxyribonucleotides in Table II, in another experiment, the acid-solubilized fraction was used.

Thus, the cd mutation caused little change in DNA synthesis. The inset, the values are obtained from the slopes in the linear region. The ratios averaged 0.78. Calculated on the basis that the thymine content of T4 DNA is 68% of thymine + HmCyt, since the Thy/HmCyt ratio was found to be 2.09 (2).

Effect of Proflavine on Deoxyribonucleotide Synthesis in cd Infection—Proflavine, an intercalating agent studied earlier (5), was administered to cultures 10 min after infection by phage cdN16, and tritium release, DNA synthesis, and the Thy/HmCyt ratios were measured. Fig. 3 shows the inhibition by proflavine of tritium release from [5-3H]uridine (Fig. 2). In the inset, the values are obtained from the slopes in the linear region. The ratios averaged 0.78. Calculated on the basis that the thymine content of T4 DNA is 68% of thymine + HmCyt, since the Thy/HmCyt ratio was found to be 2.09 (2).

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release from administered [5-\textsuperscript{3}H]uridine. Proflavine added at 10 min to a final concentration of 3 \( \mu \)g/ml inhibited DNA synthesis to 10% of the control. At the same time, \( \text{H} \) release decreased to about 40%. Under these conditions, the Thy/HmCyt ratio decreased from an average of 0.78 over the period of 15 to 45 min to 0.62 on treatment by proflavine (Table III). Since the ratio changed only minimally, proflavine must have inhibited both dTMP and HmdCMP formation about equally.

The inability of proflavine to cause an increase in the ratio in phage T4 cd infection is expected if the effect of proflavine occurs at a step prior to the formation of dCMP and dUMP. Thus the activity of dCMP deaminase is necessary to increase the ratio above about 0.6. These results fit the suggestion made earlier that the action of proflavine might be at ribonucleoside diphosphate reductase (5).

**DISCUSSION**

In an earlier study, we suggested that the phage-induced enzyme, dCMP deaminase, does not seem to be regulated in vivo by its feedback inhibitor dTTP (2), although obviously HmdCTP (or dCTP) is required for its activation (14). Nevertheless, while the enzyme may not normally act as a sensitive regulatory factor, the present studies show that it provides about 40% of the dTMP synthesized by the \textit{de novo} pathway.

The ratio of synthesis of dTMP to HmdCMP is 2 to 1, respectively, on infection by phage T4D or most Dna\textsuperscript{-} phage (2). Infection by a cd phage brings about nearly the same ratio of dTMP synthesis and DNA replication as T4 phage. Yet the cd mutant and also cd dna double mutants show ratios of Thy/HmCyt deoxyribonucleotide synthesis averaging about 0.6:1. This seeming paradox arises from an overproduction of HmdCMP derivatives and is considered in the following paragraphs.

In T4D-infected cultures, tritium release from administered [5-\textsuperscript{3}H]uridine is equal to the incorporation of the label from [6-\textsuperscript{3}H]uridine into DNA (1). At the same time, on infection by most Dna\textsuperscript{-} mutants, tritium release is still maintained at the rate found in T4D infection. This observation has been taken as evidence that the limiting factor in T4 DNA replication is the rate of synthesis of the deoxyribonucleotides (1). On the other hand, in cd phage infection tritium release is considerably faster than DNA synthesis. Part of this disparity arises because deoxyribonucleotide synthesis is about 50% greater in cd infection than in wild type infection, and HmdCMP derivatives accumulate (Table II).

In a double mutant carrying lesions in the cd and dna genes, dTMP and HmdCMP synthesis continued at the same level and at the same ratio, i.e. with a ratio of approximately 0.6:1, as in cells infected by cd phage alone. With dna\textsuperscript{-} mutants, the Thy/HmCyt ratio remains at 2:1 until after 25 min, and then it rises considerably. In these cases, HmdCMP synthesis gradually ceases, apparently because of product inhibition of dCYP hydroxymethylase by HmdCMP, and only deoxythymidine derivatives are formed (2). That dCYP which is unable to form HmdCMP is shunted into the dCYP deaminase pathway so that the rate of synthesis of Thy derivatives becomes equal to the rate of synthesis of Thy + HmCyt derivatives before 25 min. However, in cd dna\textsuperscript{-} mutants in the absence of dCYP deaminase, the dCMP cannot be shunted to dUMP and dTMP and continues to form HmdCMP. Therefore, the Thy/HmCyt ratio stays at about 0.6:1. In this case, HmdCMP and dTMP synthesis continue unabated after 25 min and the ratio is not altered. It can be assumed that dCYP hydroxymethylase is not product-inhibited by HmdCMP as in dna\textsuperscript{-} mutants because dCYP is not being siphoned off through the deaminase, because dCYP and HmdCMP are competitive (14) in the dCYP hydroxymethylase reaction, and because the reaction is reversible (20).

 Cultures infected by cd mutants appear to form DNA at a normal rate. The total synthesis of dTMP and HmdCMP derivatives including those incorporated into DNA is in the ratio, respectively, of 0.6:1. However, the incorporation of dTMP and HmdCMP into T4 DNA必须 is in the ratio of 2:1. Therefore, the deoxyribonucleotides accumulating in the cd-infected cell would have to be at a ratio of much less than 0.6:1 in order to maintain an overall ratio of 0.6:1. In fact, the ratio in the deoxyribonucleotide pool in cd infections was about 0.1:1.0 (Table II) at 25 min and decreased with time. Under these circumstances, the ratio of labeled Thy/HmCyt derivatives in DNA was close to 2:1, and obviously the replication system is capable of a normal rate of DNA synthesis against a ratio of nucleotides which overall is decreased by about 50-fold at 45 min (Fig. 2).

Since DNA replication in cd phage infection must occur in the face of so unfavorable a deoxyribonucleotide ratio in the pool, the possibility of an increased error frequency in the direction of Thy \( \rightarrow \) HmCyt could occur. A study of the reversion of the well characterized r\textit{II} strains of Drake (21) in cd \textit{rII} double mutants should resolve this important question.

Fig. 1 is a summary of the reactions converting [5-\textsuperscript{3}H]uridine through the reductive pathways to deoxyribonucleotides and shows the release of \( \text{H} \) into the aqueous phase at the points of synthesis of dTMP and HmdCMP. The figures above and to the left of the arrows are the approximate rates of synthesis for the indicated reactions during linear synthesis (after 20 min) at 30\textdegree. They are based on the measured values for the rates of release of \( \text{H} \) (see next paragraph), an observed ratio of Thy/HmCyt synthesis of close to 2:1 in T4D (2), and the present findings that in cd infection the Thy/HmCyt ratio averages 0.6 and that the rate of synthesis of HmdCMP plus dTMP increases by 50%. To accommodate these facts in T4D infection, we have set the rate of synthesis of dCYP from CDP at 1.5 times that of dUMP from UDP. In this scheme, 40% of the dCYP is converted to dUMP and the ratio of dTMP/HmdCMP becomes 2:1. The figures in parentheses below and to the right of the arrows represent the rates during infection by a cd phage mutant, based on the assumption that this mutant causes an equivalent increase in the rates of both reduction pathways. As a result, HmdCMP synthesis is increased about 3-fold over the wild type rate, and the rate of formation of labeled dUMP from UDP is increased by 1.5 times, so that the rate of dTMP synthesis approaches that in wild type infection. The values arrived at

**Table III**

<table>
<thead>
<tr>
<th>Time after infection</th>
<th>Labeled Thy/HmCyt</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.72</td>
</tr>
<tr>
<td>25</td>
<td>0.83</td>
</tr>
<tr>
<td>35</td>
<td>0.76</td>
</tr>
<tr>
<td>45</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Proflavine was added at 10 min after infection to half the culture to a concentration of 3 \( \mu \)g/ml. This experiment and that of Fig. 4 were carried out using the same infected culture.
TABLE IV
Summary of effect of cd mutant on rates of synthesis of thymine and HmCyt derivatives

<table>
<thead>
<tr>
<th>Relative rates</th>
<th>Accumulated deoxyribonucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Th y</td>
</tr>
<tr>
<td>Wild type T4</td>
<td>2.0</td>
</tr>
<tr>
<td>Cd</td>
<td>1.0</td>
</tr>
<tr>
<td>Cd:DNA</td>
<td>2.0</td>
</tr>
<tr>
<td>DNA (early times)</td>
<td>2.0</td>
</tr>
<tr>
<td>DNA (late times)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Including those incorporated into DNA.
* Decreases with time (see text).
* Assumed (see text and Table II).

in Fig. 1 are in keeping with all the observations. However, because the values are in part empirically derived and because errors of ±10% are realistic as a result of variations in infection levels and in the assays, these rates are offered as first approximations.

It is important to emphasize that the values obtained in this study and in our previous papers (1-3, 5) for HmdCMP and dTMP synthesis and for DNA synthesis represent the rates of conversion of administered labeled uracil derivatives to 3HOH or to thymine or 5-hydroxymethylcytosine residues of DNA based on the specific activity of the starting compounds. Of course these values include dilution by nonlabeled ribonucleotide precursors as would arise from mRNA breakdown and possible leakage from the pathway of de novo pyrimidine ribonucleotide biosynthesis. However, host DNA breakdown does not appear to mix significantly with the channel of de novo synthesis of deoxyribonucleotides and of DNA (1, 4).

Table IV presents a summary of the effects of the cd mutation on the rates of synthesis of dTMP and HmdCMP derivatives compared to the effects caused by T4D and DNA phage. It is not clear why cd phage have lower phage bursts (11) since the rates of dTMP synthesis and of DNA replication do not appear to be affected significantly. Possibly the disengaged control of the deoxyribonucleotide-synthesizing complex also has an effect on phage assembly (see paragraph below).

In a previous paper, it was shown that a mutant of gene 1, the structural gene for HmdCMP/dGMP/dTMP kinase, caused a dramatic decrease in the ratio of Thy/HmCyt deoxyribonucleotide synthesis (2). Since, to be activated, dCMP deaminase requires HmdCTP or dCTP (which is rapidly destroyed by phage-induced dCTPase), the enzyme was not considered to be fully activated on infection by the HmdCMP kinase mutant (2). This apparent decrease in dCMP deaminase activity did not lead to an increase in the ratio of *H* release from administered [5-3H]uridine as occurs with cd mutants (Fig. 2).

Proflavine was recently shown to inhibit not only DNA replication but also the synthesis of pyrimidine deoxyribonucleotides. Ultimately it inhibits HmdCMP more than dTMP synthesis so that the Thy/HmCyt ratio rises (5). However, in the original study it was not clear whether this change in ratio was caused by a block at dCMP hydroxymethylase, as in Dna- mutants, or prior to the formation of dCMP and dUMP, most reasonably at the level of ribonucleoside diphosphate reductase. The effect of proflavine on cd phage-infected cultures supports the conclusion that this intercalating agent inhibits at a step prior to dCMP and dUMP (5). Since the compound did not increase the Thy/HmCyt ratio significantly above 0.6, dCMP and dUMP synthesis are inhibited approximately equally.

Consideration should be given to the role of dCMP deaminase in the deoxyribonucleotide-synthesizing complex proposed by this laboratory (see the introduction). Based on release of *H* from administered [5-3H]uridine, introduction of the cd mutation increases the combined rates of synthesis of dTMP plus HmdCMP via the reductive pathway by about 50% over the wild type rate. It is reasonable that dCMP deaminase would be part of the complex because of its central role in dTMP synthesis. If the enzyme were in a "geared" position in the complex, its removal could lead to a disengaged, greater rate.

While dCMP deaminase has an important quantitative role in dTMP synthesis, the fine adjustment of the ratio of synthesis of Thy/HmCyt derivatives at 2:1:1 has to be sought in another control than feedback inhibition. This precise regulation may be provided through a structural control by template DNA (5). Thus it is possible that both dCMP deaminase and T4 DNA interact with ribonucleoside diphosphate reductase (5) to contribute to such a regulation.

For the dCMP deaminase plays an important role in dTMP synthesis in eukaryotic cells (25, 26). In this respect, evidence for complexes of deoxyribonucleotide-synthesizing enzymes associated with replication enzymes in animal cells have been reported by Baril and Ellford and co-workers (25). Firth and co-workers have described a membrane-associated complex of deoxyribonucleotides and DNA-synthesizing enzymes in Pneumococci (25).

E. coli does not show dCMP deaminase activity. Accordingly, the evolution of dCMP deaminase in T-even phage-infected systems should be considered. Obviously because of the requirement of dCTPase in the phage system, the host dCTP deaminase pathway to dUTP (27) is negated, and dCMP deaminase provides a replacement shunt mechanism. However, the present work suggests that this enzyme also plays some special structural or regulatory role in the deoxyribonucleotide synthetase-DNA replication complex.

REFERENCES

* C. S. Chiu, in manuscript.

5 Recently an aggregate containing many of the proteins of deoxyribonucleotide synthetase and of the DNA replication apparatus has been isolated from T4-infected cultures (22). A large percentage of the dCMP deaminase activity has been found in this complex. Unpublished experiments, this laboratory.
dCMP Deaminase in Phage T4 Deoxyribonucleotide Synthesis

Role of deoxycytidylate deaminase in deoxyribonucleotide synthesis in
bacteriophage T4 DNA replication.

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