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Direct Participation of dCMP Hydroxymethylase in Synthesis of Bacteriophage T4 DNA

(5-hydroxymethyl dCMP kinase/DNA replication/permeable cells)

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ABSTRACT **In order to retain in an *in situ* system the control mechanisms involved in synthesis of bacteriophage T4 DNA, infected cells were made permeable to nucleotides by plasmolysis with concentrated sucrose. Such preparations use exogenous deoxyribonucleotides to synthesize T4 phage DNA. As has been observed with *in vivo* studies, DNA synthesis was drastically reduced in plasmolyzed preparations from cells infected by *amber* mutants of genes 1, 32, 41, 42, 43, 44, or 45. Added 5-hydroxymethyl dCTP did not bypass either a mutant of gene 42 (dCMP hydroxymethylase) or of gene 1 (phage-induced deoxyribonucleotide kinase). In a phage system lacking deoxycytidine triphosphatase (gene 56) and the gene-46 product, and therefore incorporating dCTP into DNA, dCTP incorporation did not require dCMP hydroxymethylase, in keeping with *in vivo* results. With a triple *amber* mutant of genes 1, 46, and 56 only slight incorporation of dCTP occurred. By contrast, in experiments performed *in vivo* the synthesis of cytosine-containing DNA was unaffected by an *amber* mutation in gene 1.**

These studies provide evidence that dCMP hydroxymethylase, in addition to its known catalytic function, has a second, more direct, role in phage T4 DNA synthesis, apparently in recognition of hydroxymethyl dCTP. The role of the phage-induced deoxyribonucleotide kinase in T4 DNA synthesis in the plasmolyzed system remains unresolved.

While the work of several laboratories (1–5) has established the intimate role of phage T4-induced DNA polymerase in the synthesis of T4 DNA, it has long been clear that several other factors are required. For example, DNA ligase (gene 30) (6, 7), an unwinding protein (gene 32) (8), and other undefined components participate in the process (9, 10). Clearly, DNA synthesis in *Escherichia coli* requires several factors as well (11).

As a result, the concept that a complex of enzymes or structural components is required for DNA synthesis both in phage T4 infection and in bacterial systems has been conconsidered (9, 11–13). Alberts and coworkers, using a concentrated extract prepared from T4-infected cells, have shown a requirement in DNA synthesis for the products of genes 41, 44, 45, and 62 (14). An interaction has been found between the products of genes 44 and 62 (15) and also between phage T4 DNA unwinding protein (gene 32) and T4 DNA polymerase (gene 43) (16).

Though the apparent involvement of the undefined *DO* (no DNA synthesis) gene products has been widely recognized, it has been generally considered that DNA synthesis proceeds quite independently of the pathways leading to its deoxyribonucleotide precursors, except insofar as these pathways become limiting (Fig. 1). On the basis of an investigation of a temperature-sensitive mutant, Chiu and Greenberg suggested that the phage T4-induced enzyme, dCMP hydroxymethylase (gene 42), in addition to catalyzing the formation of 5-hydroxymethyl dCMP, also plays a direct role in DNA replication (13). We now have tested this possibility in phage-infected cells rendered permeable by plasmolysis with sucrose (17, 18). The present communication reports that plasmolyzed preparations of T4-infected *E. coli*, referred to as the *in situ* system, incorporate exogenous nucleotides into phage DNA and provides evidence for a direct role of dCMP hydroxymethylase in this process and a possible role of phage-induced deoxyribonucleoside monophosphate kinase (5-hydroxymethyl dCMP, dTMP, dGMP; gene 1). Mutant systems lacking the kinase also were examined *in vivo* and showed profoundly different results from the *in situ* system.

MATERIALS AND METHODS

Materials. ^{3}H-Labeled deoxyribonucleotides were obtained from Schwarz–Mann Bioresearch. ^{32}P-Labeled dGMP, dTMP, dAMP, and 5-hydroxymethyl dCMP were isolated by the method of Hurlbert and Furlong (19) from bacteriophage T2 DNA that had been extracted by the procedure of Freifelder (20). These phage were grown in TCG medium (21) supplemented with 50 μCi/ml of carrier-free [^{32}P]orthophosphate (New England Nuclear Corp.). 5-hydroxymethyl dCMP was synthesized enzymatically by R. L. Somerville (22). 5-hydroxymethyl dCTP was provided by Dr. R. Wickner. The authenticity of this compound was verified by paper chromatography, by its ultraviolet absorption spectrum, and by its ability to support DNA synthesis in a soluble extract of a culture infected by *amB24*, a kinase$^-$ mutant (see *Results*).

Biological Materials. E. coli B was originally obtained from J. Spizizen (32) and is a Su$^-$ strain. *E. coli* K12 strain D110 (*polA*$_1$ *endI*$^-$ *thy*$^-$) was provided by R. Wickner. The phage strains used are described in Table 1. All mutant strains were derived from phage T4D, except *td8*, a derivative of T4B. The mutant *amN122* had been purified by John Wiberg by crossing three times against T4D, and did not contain the 'm' mutation in gene 43 that was present in the original *amN122* isolate (2). The presence of an unaltered gene 43 in *amN122* was further verified by the demonstration that *amN122* and *amB22* complemented each other in phage production. Extracts of cells infected by *amN122* also showed normal DNA

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethyl ether) *N,N'*-tetraacetic acid.

polymerase activity. A double mutant containing a defect in gene 46 (*amN130*) in addition to a gene-56 lesion (*amE56*) was constructed by a standard phage cross in *E. coli* CR63, a Su^+ strain (10), and isolated by the procedure of Wiberg (24). This double mutant was then crossed with *amN122* to obtain the triple mutant, *amE56–amN130–amN122*. Similarly, a triple mutant *amB24–amE56–amN130* was constructed by crossing this double mutant with *amB24*.

Preparation of Plasmolyzed, Phage-Infected Cells. Cells were grown in the medium of Fraser and Jerrel (25) at 37° to a density of 5×10^8/ml and then infected with a multiplicity of 8–10 phage per bacterium. The infection was terminated after 10 min by pouring the culture over cracked ice and harvesting the infected cells by centrifugation at 4° and 6000 × *g* for 5 min. The pelleted cells were suspended at 0° in 0.01 volume of the plasmolysis buffer, containing 2 M sucrose, 0.01 M EGTA, and 0.04 M Tris·HCl (pH 8.1) as described by Wickner and Hurwitz (18), and maintained at 0°. The preparation could be kept for at least 4 hr without loss of activity but did not withstand freezing.

Assay of DNA Synthesis. DNA synthesis in plasmolyzed cell preparations was measured as incorporation of ^3H- or ^{32}P-labeled deoxyribonucleotides into acid-precipitable, alkali-stable material. The reaction mixture contained in a final volume of 0.1 ml: 3.2 μmol of Tris·HCl (pH 8.4), 0.16 μmol of Mg acetate, 0.16 μmol of EGTA, 40 μmol of sucrose, 6.4 μmol of KCl, 100 nmol of ATP, 10 nmol of each of the substrate deoxyribonucleotides (either mono- or triphosphates including one radioactively labeled with a specific activity of 10–30 cpm/pmol), as described in the legends to the figures and Table 2, and 5×10^8 plasmolyzed cells. Reaction mixtures were incubated in a 37° water bath, and the reaction was stopped by addition of 0.5 ml of 0.5 M NaOH–1% sodium dodecyl sulfate. Tubes containing the mixtures were then heated at 80° for 15 min, followed by addition of 4 ml of cold 10% trichloroacetic acid. After it was chilled, the precipitated material was collected on nitrocellulose filters (Schleicher and Schuell B-6, 2.4 cm) and counted (26). DNA synthesis, as nucleotide equivalents, was calculated from incorporation of labeled dNTP or dNMP, based on an AT/GC ratio for phage T4 DNA of 1.89 (27). The rate of synthesis was proportional to the number of plasmolyzed cells in the range used. Incorporation of deoxyribonucleoside triphosphates in extracts was measured as described by Goulian *et al.* (1).

TABLE 1. *Bacteriophage T4 mutants*

Gene	Mutant	Phenotype	Defect	Source
1	*amB24*	DO	Kinase	a
1	*amC42*	DO	Kinase	b
1	*amE957*	DO	Kinase	b
32	*amA453*	DO	DNA unwinding protein	a
41	*amN81*	DS	Uncharacterized	a
42	*amN122*	DO	dCMP hydroxymethylase	b
42	*tsL13*	DO	dCMP hydroxymethylase	a
43	*amB22*	DO	DNA polymerase	a
44	*amN82*	DO	Uncharacterized	a
45	*amE10*	DO	Uncharacterized	a
46	*amN130*	DA	Degradation of host DNA	a
56	*amE56*	DO	dCTPase	a
td	*td8*	Thy^-	dTMP synthetase	c
–	*T4D*	Wild-type		a
46–56	*amN130–amE56*			d
42–46–56	*amN122–amN130–amE56*			d
1–46–56	*amB24–amN130–amE56*			d

DO, no DNA synthesis; *DS*, some DNA synthesis; *DA*, DNA arrest. (*a*) R. S. Edgar; (*b*) J. S. Wiberg; (*c*) I. Tessman (28); (*d*) this study.

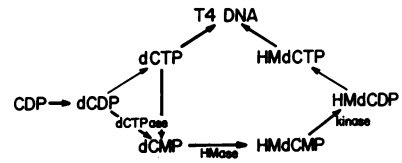

FIG. 1. Pathways of synthesis of dCTP and 5-hydroxymethyl (*HM*)dCTP after infection with phage T4.

Enzyme Assays. As a criterion of successful and comparable infection (28), we have routinely measured dCMP hydroxymethylase and dTMP synthetase activities by methods described (26, 29), and in all but one of the experiments reported here, comparable infection occurred. In the one exception a small correction was made (see legend to Fig. 5).

RESULTS

Phage T4-infected, plasmolyzed preparations form phage DNA from deoxyribonucleotides

Wickner and Hurwitz (18) presented data showing that plasmolyzed *E. coli* cells use deoxyribonucleoside triphosphates for DNA synthesis. We have used T4-infected cells plasmolyzed as described by these workers but assayed for DNA synthesis under the somewhat altered conditions that are optimal for this system (manuscript in preparation). Such

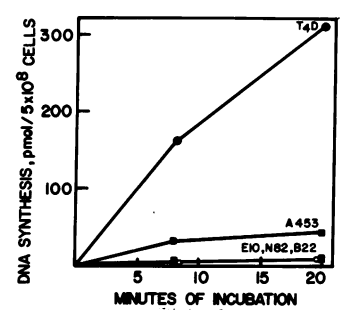

FIG. 2. DNA synthesis in cells plasmolyzed by sucrose treatment after infection by various phage T4 mutants. The cells were grown, infected, plasmolyzed, and assayed for DNA synthesis as described in *Methods*. In addition to ATP and the other components, the reaction contained 10 nmol each of dGMP, dTMP, 5-hydroxymethyl dCMP, and [^{32}P]dAMP (16 cpm/pmol). The phage used were: T4D, *am453* (gene 32), *amE10* (gene 41), *amB22* (gene 43), and *amN82* (gene 44).

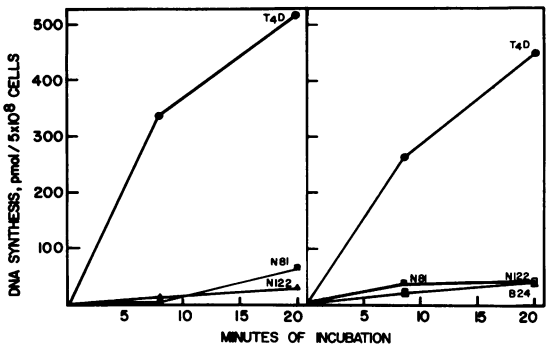

FIG. 3 (*left*). 5-hydroxymethyl [^{32}P]dCMP incorporated into DNA of plasmolyzed cells infected by T4D but not after infection by *amN122* (dCMP hydroxymethylase). Plasmolyzed, phage-infected cells were prepared and assayed for DNA synthesis as in the legend to Fig. 2, but with 10 nmol each of dGMP, dTMP, dAMP, and [^{32}P]hydroxymethyl dCMP (16 cpm/pmol). *amN81* is a mutant of gene 41.

FIG. 4 (*right*). Failure to obtain DNA synthesis by use of 5-hydroxymethyl dCTP to bypass mutations in gene 42 (dCMP hydroxymethylase) or in gene 1 (kinase). Cells infected with T4D, *amN122*, *amB24*, and *amN81* were plasmolyzed and assayed as in the legend to Fig. 2 but with 10 nmol each of dATP, dGTP, hydroxymethyl dCTP, and [^{3}H]dTTP (12 cpm/pmol).

phage-infected preparations incorporate base-labeled nucleotides, including dTTP and dATP, into DNA, and the resulting DNA synthesis is partially dependent on exogenous nucleotides (manuscript in preparation). ^{32}P-Labeled 5-hydroxymethyl dCMP, dGMP, dAMP, and dTMP are each incorporated as such into DNA, defined as acid-precipitable, hot alkali-stable, and DNase-sensitive material.

As seen in Fig. 2, the rate of incorporation of exogenous mononucleotides into DNA in a reaction system containing plasmolyzed cells infected with phage bearing *amber* mutations in genes 32, 43, 44, or 45 is greatly reduced compared to cells infected with wild-type phage T4D. It is clear from the results obtained on infection by the *amber* mutants that little or no background bacterial DNA synthesis occurred. The slight formation of DNA observed in these instances, in contrast to the absolute shut-off observed *in vivo*, might be ascribed to synthesis directly from deoxyribonucleoside triphosphates by DNA polymerase that was liberated or freed of regulation on lysis or damage of the preparation.

Identical preparations from cells infected with phage containing a mutation in the gene coding for thymidylate synthetase (*td8*) gave levels of incorporation approximately equivalent to those obtained with wild-type T4, as is expected since this mutation can be bypassed by the host dTMP synthetase. In comparable experiments with phage lacking gene-56 product (*amE56*) and with 5-hydroxymethyl dCMP or 5-hydroxymethyl dCTP plus the other nucleotides, the DNA synthesis was 1/3–1/2 of that shown by the T4D control (see below). While the data reported in this study were obtained with infected *E. coli* B, we have observed identical results using *E. coli* D110 (*polA*$_1$ *endI*$^-$) as the host bacterium. Thus, the presence of DNA polymerase I is not a complicating factor in this system. However, plasmolyzed preparations of phage-infected D110 are significantly less stable than those made from infected *E. coli* B cultures.

Experiments to be presented elsewhere have shown that DNA is made within the plasmolyzed particle, as isolated by sucrose-gradient centrifugation, and not by enzymes secreted into the medium. In addition, labeled DNA formed in plasmolyzed preparations remains within the particle. This DNA corresponds to the size and density of mature phage DNA, as determined by sucrose-gradient and CsCl equilibrium density measurements (manuscript in preparation).

When [*5*-^{3}H]dCMP is used as the radioactive precursor, essentially no net incorporation of label into DNA occurs with T4-infected cells. dCMP labeled in the 5 position obligatorily loses its tritium when it is converted to 5-hydroxymethyl dCMP (26). Quantitatively, the ^{3}H released in this system corresponds closely to the 5-hydroxymethyl dCMP formation required to support the observed level of DNA synthesis. [*5*-^{3}H]dUMP formed from dCMP or dCTP, of course, also loses its ^{3}H on being converted to dTMP (29). Hence, the phage-induced pathway that exists *in vivo* for synthesis of the virus-specific hydroxymethyl deoxycytidine nucleotides is operable in these cells. This was confirmed by the finding that the product DNA contained hydroxymethylcytosine in place of cytosine (manuscript in preparation). We take these results as evidence that this *in situ* system replicates phage DNA and, in contrast to synthesis in extracts, is dependent on *DO* and *DS* (some DNA synthesis) genes, as required *in vivo*.

Gene 42 (hydroxymethylase$^-$) and gene 1 (kinase$^-$) amber mutants are unable to incorporate 5-hydroxymethyl dCTP into DNA in plasmolyzed cells

T4-infected plasmolyzed cell preparations will readily incorporate preformed 5-hydroxymethyl [^{32}P]dCMP into DNA (Fig. 3). By contrast, cells infected with *amN122*, a mutant blocked in the ability to convert dCMP to 5-hydroxymethyl dCMP show negligible incorporation of the hydroxymethyl dCMP under these same conditions. This same result was observed in reactions containing [^{3}H]dTTP or [^{3}H]dTMP plus unlabeled 5-hydroxymethyl dCMP and the other nucleotides (data not shown).

Furthermore, this block could not be bypassed by supplying 5-hydroxymethyl dCTP (Fig. 4). This figure also shows that preparations of cells infected with *amB24*, a mutant unable to form the phage-specific deoxyribonucleotide kinase, failed to synthesize DNA even when provided with preformed triphosphates including 5-hydroxymethyl dCTP. [Mutants *amC42* (backcrossed three times against wild-type phage) and *amE957*, both in gene 1, also showed no detectable DNA synthesis.] This observation was verified with [^{3}H]dATP as the label in the presence of 5-hydroxymethyl dCTP, dTTP, and dGTP.

In contrast to the plasmolyzed system, in a crude extract of a culture infected by *amB24*, 5-hydroxymethyl dCTP supported DNA synthesis, with [^{3}H]dTTP as the label. At the same time neither dCTP (because of dCTPase) nor 5-hydroxymethyl dCMP allowed DNA synthesis.

Gene-42 mutant incorporates labeled dCTP into DNA in plasmolyzed cells, but gene-1 mutant decreases the incorporation greatly

Wiberg (24) has shown that infection with a phage carrying amber mutations in gene 56 (dCTPase$^-$), which prevents the breakdown of dCTP, and gene 46 or 47, which stabilizes cytosine-containing DNA, resulted in the synthesis of a

limited but significant amount of DNA containing cytosine in place of hydroxymethylcytosine (30, 31). Furthermore, it was found that a triple mutant with a lesion in gene 42, in addition to mutations in genes 56 and 46, showed the same level of synthesis (32).

The finding with this triple mutant could be a strong argument against the idea put forward by Chiu and Greenberg (13) that dCMP hydroxymethylase has a second role in phage DNA synthesis. However, it was suggested that dCTP might enter DNA without the participation of dCMP hydroxymethylase (13). In the following experiment it is clear that, in fact, dCTP incorporation does not require dCMP hydroxymethylase.

Table 2 shows the levels of incorporation of 5-hydroxymethyl [^{32}P]dCMP and [5-^{3}H]dCTP observed in plasmolyzed preparations of cells infected with a mutant containing lesions in genes 56, 46, and 42, in genes 56 and 46, or in gene 42 alone. These data again show that the ability to incorporate significant amounts of preformed 5-hydroxymethyl dCMP is specifically dependent on the presence of the product of gene 42. However, a mutation in this gene has no effect on the synthesis of DNA from [^{3}H]dCTP, in accordance with the *in vivo* studies of Kutter and Wiberg (32). Table 2 also indicates that dCTP is not incorporated in the presence of dCTPase and an active product from gene 46.

In other studies we have found that the triple mutant (42–56–46) and the double mutant (56–46) incorporate [5-^{3}H]-dCMP into DNA readily (not shown). Since the host dCMP kinase and the general deoxyribonucleoside diphosphate kinase form dCTP (33), it is to be expected that dCTPase-deficient systems will use [5-^{3}H]dCMP for DNA synthesis.

Table 2 also shows that the rate of incorporation of labeled dCTP into DNA in plasmolyzed preparations of cells infected with a mutant containing a defective gene-1 product is greatly reduced, even in the presence of the mutations in genes 56 and 46. Thus, the kinase enzyme is required not only for 5-hydroxymethyl dCTP incorporation (Fig. 4) but for maximum incorporation of dCTP. In experiments with dCTPase-deficient systems, the level of DNA synthesis was only about 1/3–1/2 that of a wild-type control, in keeping with observations made *in vivo* (24, 32). It should be emphasized however, that the values obtained for the incorporation of dCTP in the triple mutant (1–46–56) are, in fact, only slightly, though still significantly, above the range of background levels observed with other *DO* amber mutants.

TABLE 2. *Differential effects shown by gene-42 product (dCMP hydroxymethylase) and gene-1 product (kinase) on incorporation of labeled dCTP into phage DNA*

Mutant gene	Labeled precursor	
	[5-^{3}H]dCTP (pmol)	[^{32}P]Hydroxymethyl dCMP (pmol)
56,42,46	186	36
56,46	186	156
42	36	24*
56,1,46	60	—
1	24	—
T4D	18	510

The values represent the DNA synthesized in 20 min at 37° by plasmolyzed cells infected and assayed as in the legend to Fig. 2 but with 10 nmol each of dGMP, dAMP, dTMP, and either [5-^{3}H]dCTP (18 cpm/pmol) or [^{32}P]hydroxymethyl dCMP (13 cpm/pmol).

* In several other assays, preparations of cells infected by amber mutants of genes 41, 43, 44, or 45 showed a range of 10–40 pmol; refer to comments in *Results* and see figures.

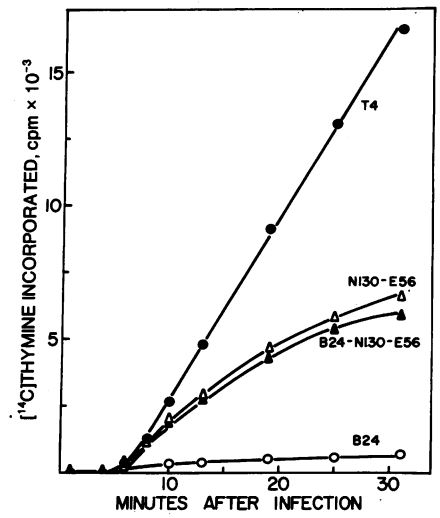

FIG. 5. *In vivo* synthesis of cytosine-containing DNA in cultures infected by a multiple *am* mutant (genes 1, 46, and 56). *E. coli* B strain 201 (*thyA, deoB*, see ref. 23) was the host and DNA synthesis was monitored by incorporation of [^{14}C]thymine ($1.67 \times \times 10^{6}$ cpm/μmol), as described earlier (13). The values for the double mutant (*amN130 amE56*) were multiplied by 1.7 to account for differences in extent of infection as measured by dCMP hydroxymethylase activity at 10 min (see *Enzyme Assays*).

In vivo the amber mutant of gene 1 has no effect on synthesis of cytosine-containing DNA

While plasmolyzed preparations of cells infected by a phage carrying amber lesions in genes 1, 46, and 56 showed very little incorporation of labeled dCTP into DNA as compared to the values obtained with a 46–56 double mutant, the results of an *in vivo* study were diametrically opposite. Fig. 5 shows that infection of a culture by a phage carrying *am* mutations in genes 46 and 56, and thus forming DNA containing cytosine rather than hydroxymethylcytosine, showed the same DNA synthesis as a triple mutant defective in genes 1, 46, and 56. Thus, *in vivo* a gene-1 *am* mutation had no effect on dCTP incorporation into DNA.

DISCUSSION

The major findings in this study may be summarized as follows. In an *in situ* system, i.e., a plasmolyzed, nucleotide-permeable preparation of infected cells: (*1*) 5-hydroxymethyl dCTP cannot enter into the formation of phage DNA in the absence of either the product of gene 42 (dCMP hydroxymethylase) or of gene 1 (phage-induced deoxyribonucleotide kinase). Thus, these two enzymes of the deoxyribonucleotide pathway cannot be bypassed by their products in a system that uses exogenous deoxyribonucleotides to form DNA. (*2*) In a phage system lacking dCTPase and the gene-46 product, and therefore incorporating dCTP into DNA, dCTP incorporation does not require dCMP hydroxymethylase but is severely depressed in the absence of the

kinase. (3) However, while the *in situ* system shows a marked though not an absolute dependency on gene-1 product in incorporating dCTP into DNA, *in vivo* the same amber mutant had no observable effect on the synthesis of DNA containing cytosine.

These studies provide strong argument that dCMP hydroxymethylase has a second function in DNA synthesis. Since the enzyme is not required in the incorporation of dCTP into DNA, an attractive model is that dCMP hydroxymethylase acts in concert, presumably in a complex with the remainder of the DNA-synthesizing apparatus, as a recognition system that is necessary to incorporate 5-hydroxymethyl dCTP into DNA.

By this picture dCTP would enter into DNA synthesis without a special recognition system other than the cooperative function of base pairing and DNA polymerase (34). The safeguard against the entrance of dCTP into T4 DNA would be the dCTPase. Cultures infected by a dCTPase$^-$, gene-46 product$^-$ double mutant incorporate dCTP but give rise to DNA of low molecular weight (32).

Because of the difference between the results obtained *in vivo* and *in situ* with the triple mutant of genes 1, 46, and 56, we must reserve judgement of the possible function of the kinase in DNA synthesis until other gene-1 mutants are examined. It is quite conceivable, however, that *in vivo* the polypeptide fragment formed by *amB24* (gene 1) is functional in a presumed second function, whereas in the *in situ* system the same fragment is distorted by the sucrose or is degraded.

It is clear that this plasmolyzed system will incorporate deoxyribonucleoside monophosphates into phage DNA. Furthermore, the studies of Gros and coworkers (17), Wickner and Hurwitz (18), and our laboratory have provided evidence that triphosphates enter this system. While 5-hydroxymethyl dCTP will not participate in DNA synthesis in the absence of either the product of gene 42 or of gene 1, DNA is formed in the presence of dCTP whether or not gene 42 is mutated, so long as genes 56 and 46 are also mutated (Table 2). In the absence of dCTPase no efficient pathway to convert dCTP to dCMP exists other than reversal of host kinases or breakdown of product DNA. In fact, Price and Warner (30) have shown that only about 5–10% of the total cytosine nucleotides in the DNA of a dCTPase$^-$ mutant was present as 5-hydroxymethyl dCMP, and some of the dCMP required for this synthesis was considered to be derived from subsequent breakdown of the labile cytosine-containing DNA. Therefore, all or nearly all dCTP must enter the system as such.

Finally, it is important to emphasize that several controls found *in vivo* occur in the plasmolyzed permeable system. Thus, the system does not make DNA in the presence of mutants of genes 41, 44, and 45. These controls are obviously lost in extracts but are still retained in concentrated preparations (14, 15). However, to our knowledge, such preparations are not dependent on dCMP hydroxymethylase or the kinase. Therefore, the plasmolyzed system has the possibility of gaining insight into such forms of regulation.

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1. Goulian, M., Lucas, Z. J. & Kornberg, A. (1968) *J. Biol. Chem.* **243**, 627–638.
2. De Waard, A., Paul, A. V. & Lehman, I. R. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 1241–1248.
3. Warner, H. R. & Barnes, J. E. (1966) *Virology* **28**, 100–107.
4. Speyer, J. F. (1965) *Biochem. Biophys. Res. Commun.* **21**, 6–8.
5. Drake, J. W., Allen, E. F., Forsberg, S. A., Preparata, R-M. & Greening, E. O. (1969) *Nature* **221**, 1128–1132.
6. Fareed, G. C. & Richardson, C. C. (1968) *Proc. Nat. Acad. Sci. USA* **58**, 665–672.
7. Sugimoto, K., Okazaki, T. & Okazaki, R. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 1356–1362.
8. Alberts, B. & Frey, L. (1970) *Nature* **227**, 1313–1318
9. Richardson, C. C. (1969) *Annu. Rev. Biochem.* **38**, 795–840.
10. Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, C. H. & Lielausis, E. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 375–392.
11. Gros, J. D. (1972) *Curr. Top. Microbiol. Immunol.* **57**, 39–74.
12. Goulian, M. (1971) *Annu. Rev. Biochem.* **40**, 855–898.
13. Chiu, C.-S. & Greenberg, G. R. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 351–359.
14. Barry, J. & Alberts, B. (1972) *Fed. Proc.* **31**, 442.
15. Barry, J. & Alberts, B. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2717–2721.
16. Huberman, J., Kornberg, A. & Alberts, B. (1971) *J. Mol. Biol.* **62**, 39–52.
17. Ben-Hamida, F. & Gros, F. (1971) *Biochemie* **53**, 71–80.
18. Wickner, R. B. & Hurwitz, J. (1972) *Biochem. Biophys. Res. Commun.* **47**, 202–211.
19. Hurlbert, R. B. & Furlong, N. B. (1967) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. XII, part A, pp. 193–202.
20. Freifelder, D. (1967) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. XII, part A, pp. 550–554.
21. Nomura, M., Okamoto, K. & Asano, K. (1962) *J. Mol. Biol.* **4**, 376–387.
22. Greenberg, G. R. & Somerville, R. L. (1962) *Proc. Nat. Acad. Sci. USA* **48**, 247–257.
23. Lomax, M. S. & Greenberg, G. R. (1968) *J. Bacteriol.* **96**, 501–514.
24. Wiberg, J. S. (1966) *Proc. Nat. Acad. Sci. USA* **55**, 614–621.
25. Fraser, D. & Jerrel, E. A. (1953) *J. Biol. Chem.* **205**, 291–295.
26. Lomax, M. I. S. & Greenberg, G. R. (1967) *J. Biol. Chem.* **292**, 109–113.
27. Wyatt, G. R. & Cohen, S. S. (1953) *Biochem. J.* **55**, 774–782.
28. Shapiro, D. M., Eigner, J. & Greenberg, G. R. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 874–881.
29. Yeh, Y.-C. & Greenberg, G. R. (1967) *J. Biol. Chem.* **242**, 1307–1313.
30. Price, A. R. & Warner, H. R. (1969) *Virology* **39**, 882–892.
31. Kutter, E. M. & Wiberg, J. (1969) *J. Virol.* **4**, 439–453.
32. Kutter, E. M. & Wiberg, J. (1968) *J. Mol. Biol.* **38**, 395–411.
33. Hiraga, S. & Sugino, Y. (1966) *Biochim. Biophys. Acta* **114**, 416–418.
34. Kornberg, A. (1969) *Science* **163**, 1410–1413.