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In Vivo Functional Interaction Between DNA Polymerase and dCMP-Hydroxymethylase of Bacteriophage T4

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Some mutations in the structural gene for T4 DNA polymerase (gene 43) behave as suppressors of a deficiency in T4 dCMP-hydroxymethylase (gene 42). The suppression appears to involve a functional interaction between the two enzymes at the level of DNA replication. The hydroxymethylase deficiency caused DNA structural abnormalities in replication, and DNA polymerase lesions appeared to partially reverse these abnormalities. The results do not necessarily imply protein-protein interactions between the two enzymes, although both enzymes appear to play roles in controlling the fidelity of phage DNA replication.

The results of this study bear on the issue of whether or not the DNA replication proteins of T4 phage interact with one another in vivo, e.g., form a replication protein complex. The case to be described involves a possible interaction between T4 DNA polymerase (product of phage gene 43) and T4 dCMP-hydroxymethylase (product of phage gene 42). We found that some DNA polymerase lesions lead to a suppression of the effects of a dCMP-hydroxymethylase lesion. This suppression did not appear to involve either activation or increased synthesis of dCMP-hydroxymethylase; rather, it seemed to involve an improvement by altered DNA polymerases of the quality of DNA synthesized by gene 42-defective phage. Our results are consistent with models that have proposed a direct role for the dCMP-hydroxymethylase in T4 DNA replication in addition to its role in synthesis of the T4 DNA precursor hydroxymethyl-dCMP (4, 5); however, the functional relationship of this enzyme to the T4-induced DNA polymerase may or may not involve protein-protein interactions.

MATERIALS AND METHODS

Phage and bacterial strains. The UGA mutant T4 *H4201* (gene 42) was generated by mutagenesis with hydroxylamine (prefix *H*) and was extensively backcrossed to remove secondary mutations from its genome (P. V. O'Donnell, Ph.D. thesis, Cornell University Graduate School of Medical Sciences, New York, N. Y., 1973). The prefix *N* is used to designate spontaneous nonsense mutants that were isolated in the work described herein. Other phage mutants used were originally obtained from R. S. Edgar and W. B. Wood. *Escherichia coli* strains CAJ70 (*su*$^+$UGA), W3110 (*su*^{o}), and B^E (*su*^{o}) were gifts from S. Brenner, A. Hershey, and L. Gold, respectively. The *su*^{o} strains do not harbor specific suppressors for nonsense mutations but can partially suppress such mutations by ribosomal ambiguity (10, 14). The *su*$^+$UGA strain carries a specific suppressor for UGA mutations (21). Streptomycin-resistant (*str*^{r}) derivatives of these bacterial strains were isolated as previously described (14) and exhibited reduced levels of suppression by ribosomal ambiguity.

Media and growth conditions. The synthetic medium M9S contained 42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.5 mM NaCl, 18.6 mM NH_4Cl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.01 mM $FeCl_3 \cdot 6H_2O$, 0.25% (wt/vol) vitamin-free Casamino Acids (Difco), 0.4% (wt/vol) glucose, 20 μg of L-tryptophan per ml, and 1 μg of thiamine hydrochloride per ml. M9 medium was the same as M9S, except that it did not contain Casamino Acids. Other media and conditions for plating, determination of burst size, and determination of thymidine incorporation were as previously described (14). The analysis of ^{14}C-labeled T4-induced proteins by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate was carried out as previously described (12, 13). Assays of T4 dCMP-hydroxymethylase were carried out as described by Wiberg et al. (24).

RESULTS

Properties of the T4 gene 42 mutant *H4201*. Table 1 shows some of the growth characteristics of the T4 gene 42 UGA mutant *H4201*. This mutant grew well on *E. coli* W3110 and poorly on *E. coli* B^E. The observed growth was probably due to suppression by ribosomal ambiguity, since T4 *H4201* was severely restricted on *str*^{r} derivatives of W3110 and B^E. In our studies, B^E and B^E *str*^{r} were more restrictive to T4 nonsense mutants than were W3110 and W3110 *str*^{r}, respectively. Table 1 also lists some of the growth properties of two T4 gene 43 UGA mutants that will be discussed in this report.

T4 *H4201* exhibited high frequencies of reversion on the *E. coli* W3110 *str*^{r} and B^E hosts (Table 1). All of 25 plaques that were picked

TABLE 1. *Growth properties of T4 gene 42 and gene 43 UGA mutants*

E. coli host	Plating efficiency (%)[a] of T4 mutant		
	H4201	*N0943*	*N1243*
CAJ70 (*su*$^+$UGA)	100	100	100
CAJ70 *str*^{r} (*su*$^+$UGA)	80	90	88
W3110 (*su*^{o})	80	87	89
W3110 *str*^{r} (*su*^{o})	0.025	38	78
B^E (*su*^{o})	0.4	26	36
B^E *str*^{r} (*su*^{o})	3×10^{-6}	6×10^{-5}	3×10^{-5}

[a] Dilutions of phage stocks were plated with cells grown to about 5×10^8/ml in nutrient broth according to methods described previously (14). Plaque counts were determined after overnight incubation at 30°C and ranged between 200 and 500 plaques. Plating efficiencies were determined relative to the respective phage titer on CAJ70 (*su*$^+$UGA).

TABLE 2. *Complementation tests that assigned T4 mutants N0943, N1243, and ts1043 to gene 43*[a]

Phage mutant	Growth temp (°C)	Rate of [^{3}H]thymidine incorporation (cpm $\times 10^{-3}$) in cross with T4 mutant		
		amE4301[b] (gene 43)	*amN82*[b] (gene 44)	None
N0943	30	5.3	110	6.6
N1243	30	5.8	95	13
amE4301	30		79	4.9
amN82	30			4.6
ts1043	42	4.3	282	5.8
tsL56[b]	42	2.7	250	9.1
amE4301	42		256	4.5
amN82	42			5.1

[a] Phage crosses were carried out in M9S medium on *E. coli* B^E *str*^{r}. In each case, 0.05 ml containing 2.5×10^8 plaque-forming particles of each of the coinfecting phages was added to 0.5 ml containing 5×10^7 log-phase cells. At 20 min postinfection, 0.1 ml of culture was added to 0.1 ml of M9S containing 20 μCi of [^{3}H]thymidine at a specific activity of 20 μCi of ^{3}H per μg of thymidine. Incorporation was allowed to proceed for 10 min in crosses at 30°C and for 5 min in crosses at 42°C before it was stopped by the addition of 5 ml of cold 5% trichloroacetic acid and 1 drop of 0.5% albumin as carrier. The solutions were then chilled in ice, and acid-insoluble counts were then determined as previously described (12).

[b] Mutants *tsL56* and *amE4301* map in gene 43 and *amN82* maps in gene 44 (2, 7).

from a plating of T4 *H4201* on B^E failed to grow on B^E *str*^{r} and were shown by recombination tests to have retained the *H4201* mutation. These results suggested that second-site phage mutations can lead to more efficient growth of T4 *H4201* in *E. coli* B^E cells. It will be shown that such an enhancement of growth of T4 *H4201* can be brought about by mutations in T4 gene 43.

High frequency of T4 gene 43 mutations among T4 *H4201*-pseudorevertants. Twelve plaques were picked from a plating of T4 *H4201* on *E. coli* CAJ70 (*su*$^+$UGA). These were replated on *E. coli* B^E, and one "revertant" plaque was picked for each of the 12 T4 *H4201* isolates. All appeared to be pseudorevertants of T4 *H4201*. High-titer stocks of these pseudorevertants were subsequently prepared and used for genetic and gel electrophoretic assays to identify the second-site mutations that caused efficient growth of T4 *H4201* on the *E. coli* B^E host. Among the 12 T4 *H4201*-pseudorevertants studied, two carried temperature-sensitive (*ts*) mutations at one gene 43 site (named *ts1043*) and three carried UGA nonsense mutations at two gene 43 sites (named *N0943* and *N1243*, respectively). The results of complementation tests that identified *ts1043*, *N0943*, and *N1243* as T4 gene 43 mutations are shown in Table 2. These tests were carried out on *E. coli* B^E *str*^{r}, which, as is shown in Table 1, was the least permissive host strain for the T4 UGA mutants we used. *N0943*, *N1243*, and *ts1043* failed to complement a known amber mutation in T4 gene 43 but did complement a known amber mutation in T4 gene 44. In other experiments (data not shown), it was observed that *ts1043*, *N0943*, and *N1243*, as well as the *H4201* pseudorevertants from which they were derived, complemented mutations in T4 genes 1, 30, 32, 41, 44, 45, 56, and 62. These genes are known to be involved in control of phage DNA replication (25). The other seven T4 *H4201*-pseudorevertants we isolated were not completely characterized. These appeared to be normal for all the T4 DNA replication gene functions, except for gene 42, and may have resulted from either intragenic suppression, i.e. second-site mutations within gene 42, or intergenic suppression via nondetrimental lesions in gene 43 or other phage genes.

Growth of the UGA double mutants of T4 genes 42 and 43 (T4 *H4201/N0943* and T4 *H4201/N1243*) on *E. coli* B^E, an *su*^{o} host, reflects in part the relatively high efficiency of suppression of UGA mutations by mistranslation (10, 22). In Table 3 we show that single mutants T4 *N0943* and T4 *N1243* grew efficiently on *E. coli* B^E and poorly on an *str*^{r} derivative of this *su*^{o} host. The gene 43 DNA polymerase is utilized in catalytic amounts for phage DNA replication, and low levels of enzyme can lead to large phage yields (14). Figure 1 shows the results of gel electrophoretic assays, which demonstrated that in *E. coli* B^E both *N0943* and *N1243* synthesize small amounts of normal-sized gene 43 protein.

Table 3 also shows the effects of two known T4 gene 43 mutations, *tsL42* and *tsL56*, that were isolated and characterized in other unrelated studies (2, 6). The *tsL42* mutation en-

hanced the growth of *H4201* phage, whereas the *tsL56* mutation did not. It appears then that only specific lesions in T4 gene 43 can enhance the growth of T4 *H4201*. Additional studies with T4 *H4201/ts1043* (Table 3) confirmed that the higher efficiency of growth of this pseudorevertant relative to T4 *H4201* on *E. coli* B^E was due to the gene 43 mutation. The single *ts1043* mutant was isolated from a backcross of *H4201/ts1043* and was then used to construct T4 *H4201/ts1043* double mutants in a cross with T4 *H4201*. All of three such double mutants isolated from the cross exhibited the growth characteristics of the original pseudorevertant rather than those of the T4 *H4201* single mutant when tested on *E. coli* strains B^E, B^E *str*^{r}, W3110, and W3110 *str*^{r}.

TABLE 3. *Suppression of T4 mutant H4201 (gene 42) by T4 gene 43 mutations*

Phage	Defective gene(s)	Plating efficiency (%)[a] on *E. coli*		Burst size[b] on *E. coli*	
		B^E	B^E *str*^{r}	B^E	B^E *str*^{r}
Wild type	None	96	91	380	140
H4201	42	0.7	5×10^{-6}	35	0.01
H4201/N0943	42, 43	30	$<1 \times 10^{-7}$	99	<0.01
N0943	43	44	4×10^{-5}	148	1.4
H4201/N1243	42, 43	49	$<1 \times 10^{-7}$	65	0.4
N1243	43	56	3×10^{-5}	242	29
H4201/ts1043	42, 43	83	8×10^{-6}	77	0.03
ts1043	43	76	100	163	110
H4201/tsL42	42, 43	56	5×10^{-6}	83	0.1
tsL42	43	74	50	209	125
H4201/tsL56	42, 43	2.6	1×10^{-4}	5	<0.01
tsL56	43	76	82	142	80

[a] Plating efficiencies were determined at 30°C relative to the respective phage titers on *E. coli* CAJ70 (*su*$^+$UGA). Conditions were similar to those described in Table 1.

[b] Burst size (average phage yield per infected cell) measurements were carried out at 30°C in M9S medium as previously described (14). The concentration of infected cells used in the computation of burst size was determined as the concentration of plaque-forming infective centers in the cultures infected with T4 wild type.

T4 gene 43 mutations do not specifically increase hydroxymethylase (T4 gene 42) activity. It is known that defects in T4 DNA replication and/or expression of "late" phage functions lead to a delay in the normal shutoff of "early" phage protein synthesis (11, 23). Under such conditions, several "early" enzymes, e.g., dCMP-hydroxymethylase, are synthesized to higher levels than in infections with wild-type phage. We compared the levels of T4-induced dCMP-hydroxymethylase in infections of *E. coli* B^E with various phage mutants and found that T4 gene 43 mutants did not produce more hydroxymethylase than mutants of other T4 DNA replication genes. It was also found that none of the 12 T4 *H4201*-pseudorevertants mentioned in this report produced more hydroxymethylase activity than the T4 *H4201* single mutant. Examples of the data from these studies are shown in Table 4. It appears that the enhancement of T4 *H4201* growth by gene 43 mutations is not caused by increased dCMP-hydroxymethylase activity.

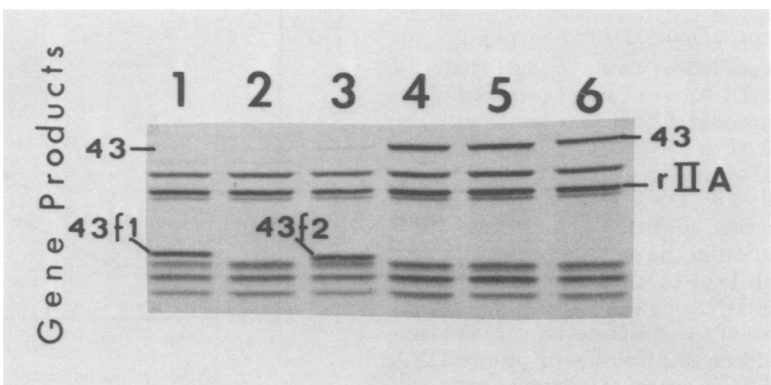

FIG. 1. *Portion of an autoradiogram showing* ^{14}C*-labeled proteins synthesized by the T4 gene 43 UGA mutants N0943 and N1243 in E. coli* B^E *(su*^{o}*). Infections were initiated by mixing 2 volumes of cell cultures at* 3×10^8 *cells per ml with 1 volume of phage solutions at* 6×10^9 *phage per ml. Growth was in a shaker bath at 30°C in M9 medium (not supplemented with amino acids). One-milliliter samples of infected cultures were each labeled with 1 μCi of a mixture of* ^{14}C*-labeled amino acids at 6 to 11 min postinfection. Extracts were subsequently prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. The concentration of acrylamide in the gel used was 7.5%. Other details of the methods used have been reported elsewhere (12, 13). Columns: 1, T4 amE4301; 2, T4 N0943; 3, T4 N1243; 4, wild-type revertant of T4 amE4301; 5, wild-type revertant of T4 N0943; 6, wild-type revertant of T4 N1243. Autoradiogram bands: 43, normal-sized gene 43 protein; 43f1, amE4301 nonsense protein fragment; 43f2, N1243 nonsense protein fragment; rIIA, protein product of the rIIA gene.*

TABLE 4. *Levels of dCMP-hydroxymethylase in T4-infected E. coli* B^{Ea}

Phage	Defective gene(s)	dCMP-hydroxymethylase (relative activity)
Wild type	None	1.0
amB24	1	4.4
amH39X	30	2.2
amHL618	32	3.5
amN81	41	3.7
amN82	44	4.6
amE10	45	4.4
amE1140	62	3.7
amE4301	43	3.7
N0943	43	3.0
N1243	43	2.6
amC87	42	0.06
amN122	42	0.01
H4201	42	0.2
H4201/N0943	42, 43	0.2
H4201/N1243	42, 43	0.2

[a] Growth was at 30°C in M9S medium, and infections were carried out as described for Table 2. At 60 min postinfection, 1-ml samples were removed and assayed for dCMP-hydroxymethylase at 30°C according to the methods described by Wiberg et al. (24). Enzyme activities were determined relative to a wild-type revertant of T4 *H4201* (820 ^{3}H cpm released from [5-^{3}H]dCMP in 60 min under conditions of the assay).

T4 gene 42 and 43 functions may interact at the level of DNA synthesis. Figure 2 shows the results of an experiment in which we measured thymidine incorporation in *E. coli* B^E cultures that had been infected with T4 *H4201* and some of its derivatives. In contrast to the observation that T4 *H4201/ts1043*-infected B^E cultures produced more viable phage than T4 *H4201*-infected cultures (Table 3), thymidine incorporation proceeded at higher levels with the single mutant (Fig. 2). These results suggested that T4 *H4201* synthesizes defective phage DNA in *E. coli* B^E, i.e., DNA that is not used for phage production, and that the presence of a gene 43 mutation in the genetic background for T4 *H4201* can lead to an improvement in the quality of the DNA synthesized by this phage.

Interaction of the T4 gene 42 and 43 functions may affect the fidelity of phage DNA replication. Two types of observations indicated that the T4 gene 42 lesion *H4201* leads to structural abnormalities in intracellular phage DNA and that some T4 gene 43 lesions prevent or correct these abnormalities.

(i) T4 *H4201*-infected cells hyperproduced the protein product of phage gene 32, whereas none of the T4 *H4201*-pseudorevertants studied exhibited 32-protein hyperproduction. Examples are shown in Fig. 3. Other work (9, 16, 20) has shown that T4 gene 32 is under autogenous control and that 32-protein hyperproduction can result from defects in gene 32 and/or defects in phage DNA replication that lead to accumulation of single-stranded DNA, which strongly binds 32-protein (1) and presumably decreases the probability of further binding of this protein to the site of autogenous control. Several genetic experiments indicated that the T4-*H4201* phage strains we used did not harbor gene 32 mutations. In one experiment, for example, the T4 *H4201* single mutant was isolated as a recombinant from a backcross of T4 *H4201/ts1043* (a double mutant that does not hyperproduce the 32-protein; Fig. 3) and was observed to resemble the original T4 *H4201* strain in 32-protein hyperproduction. T4 gene 43 protein synthesis is also known to be autogenously regulated (19), and in Fig. 3 it can be seen that the gene 43 mutations *ts1043* and *N1243* lead to hyperproduction of the corresponding defective gene products.

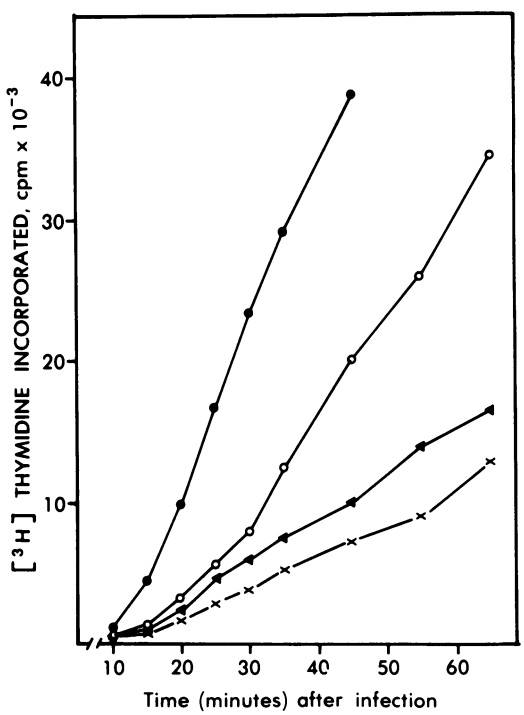

FIG. 2. *Thymidine incorporation in E. coli* B^E *infected with T4 mutants defective in genes 42 and 43. Infections were carried out as described for Fig. 1. At 5 min postinfection, 0.9 ml of each culture was added to 0.1 ml of M9 containing 20 μCi of [methyl-*^{3}*H]thymidine at a specific activity of 2 μCi of* ^{3}*H per μg of thymidine. Samples (0.1 ml) were withdrawn at the indicated times and assayed for trichloroacetic acid-insoluble* ^{3}*H as described previously (14). Symbols:* (●) *T4 wild type;* (○) *T4 ts1043;* (▲) *T4 H4201;* × *T4 H4201/ts1043.*

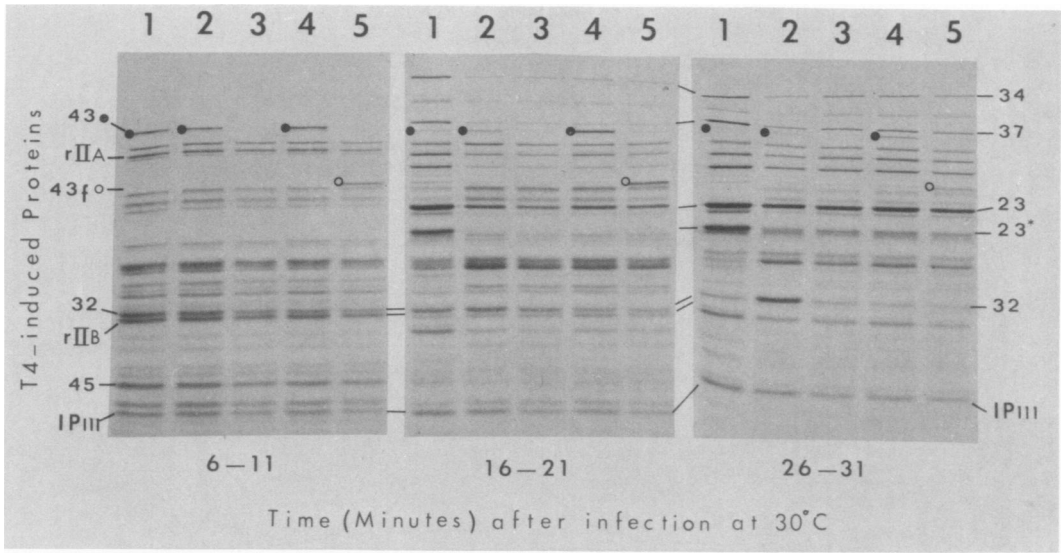

FIG. 3. *Hyperproduction of the T4 32-protein by T4 Mutant H4201 (gene 42) in E. coli B^E. Conditions were similar to those described in Fig. 1. Electrophoresis was carried out on a three-step gradient gel as previously described (13). The concentrations of acrylamide in the gradient were 7.5% (2 ml), 10% (4 ml), and 12.5% (4 ml). Columns: 1, T4 wild type; 2, T4 H4201; 3, T4 H4201/N0943; 4, T4 H4201/ts1043; 5, T4 H4201/N1243.*

(ii) Alkaline sucrose gradient analyses of DNA from cultures infected with T4 *H4201* and its pseudorevertants suggested that the gene 42 lesion leads to an abnormal accumulation of interruptions (gaps or single-stranded branching at nicks) in replicating phage DNA. Some results are shown in Fig. 4. The bulk of alkali-denatured DNA from T4 *H4201*-infected cells sedimented at a slower rate than DNA from T4 *ts1043*-infected cells. The sedimentation pattern for DNA from T4 *H4201/ts1043*-infected cells resembled that of the DNA from T4 *H4201*-infected cells; however, a significant portion of the T4 *H4201/ts1043* DNA appeared to sediment in regions of the gradient that corresponded to the sedimentation range for DNA from T4 *ts1043*-infected cells. Similar small differences in DNA sedimentation profiles between T4 *H4201* and T4 *H4201/ts1043* were observed in two additional experiments involving long-term labeling of intracellular phage DNA (results not shown).

DISCUSSION

In T4-infected *E. coli* the gene 42 dCMP-hydroxymethylase catalyzes the conversion of dCMP to 5-hydroxymethyl-dCMP (8). A phage-induced deoxyribonucleotide kinase (gene 1) converts the monophosphate to the diphosphate (3), and bacterial kinases convert the diphosphate to the triphosphate. 5-Hydroxymethyl-dCTP is presumably the immediate precursor of the hydroxymethyl cytosine residues in T4 DNA. In addition to its role in DNA precursor biosynthesis, the gene 42 hydroxymethylase may play a direct role in phage DNA replication (4, 5, 27). One model that has been suggested by Greenberg and co-workers (5; personal communications) proposes that shortly after infection the hydroxymethylase becomes part of the DNA replication complex, which includes DNA polymerase and other T4-induced proteins. The complex is thought to regulate both the cellular levels and the entry of certain DNA precursors into replicating phage DNA. This model is supported by two types of observations. (i) Many T4 gene 43 (DNA polymerase) mutants appear to be also partially defective in the formation of 5-hydroxymethyl-dCMP (5), and (ii) in T4-infected cells made permeable to 5-hydroxymethyl-dCTP, the triphosphate is not used for phage DNA synthesis if either gene 42 or gene 1 (kinase) is defective; i.e., both the hydroxymethylase and the kinase appear to be required for precursor incorporation (27). Some studies also suggest that input phage DNA is a functional part of the complex (G. R. Greenberg and J. B. Flanegan, Fed. Proc. **35**:1492, 1976).

The genetic studies that were reported here also support the notions that the gene 42 hydroxymethylase plays a direct role in phage DNA replication and that it may interact with the gene 43 DNA polymerase. It is still unclear, however, if protein-protein associations are involved in this interaction or if the two enzymes control separate steps in the same pathway. We offer two models to explain the suppression of

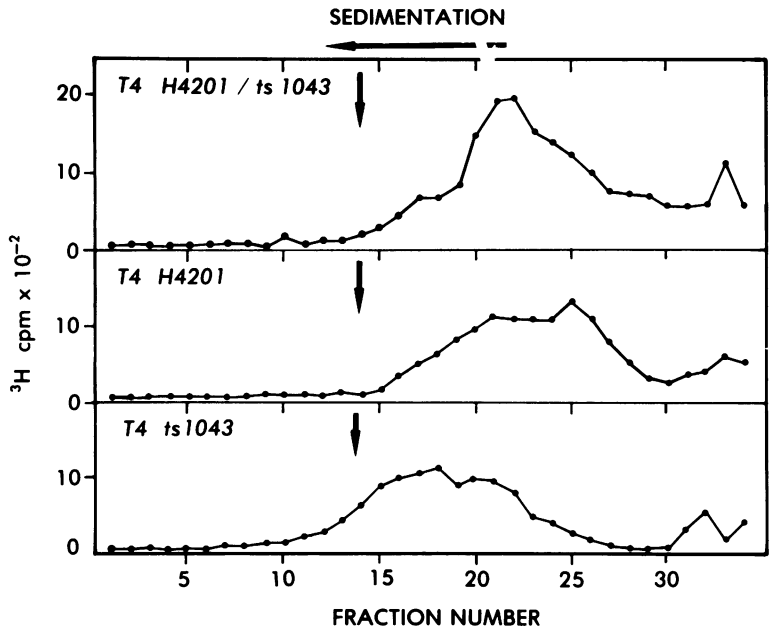

FIG. 4. *Sucrose gradient analyses of ^{3}H-labeled DNA from E. coli B^E cultures infected with T4 mutants H4201, H4201/ts1043, and ts1043. Infections were carried out as described for Fig. 1. Final volumes of infected cultures were 4.5 ml each. At 10 min postinfection, 0.1 ml of [methyl-^{3}H]thymidine was added to each culture to yield a final concentration of 20 μCi of ^{3}H per ml at a specific activity of 2 μCi of ^{3}H per μg of thymidine. ^{3}H incorporation was stopped 25 min later, and the DNA was analyzed on alkaline sucrose gradients by using the methods described by Konrad and Lehman (15), except that 2% Sarkosyl NL97 was added to the samples during the alkali treatment and the gradients contained 0.1% Sarkosyl. ^{14}C-labeled T4 DNA was used as a marker (vertical arrows) in the gradients. The source of the DNA was a purified preparation of T4 H4201 phage that was grown on CAJ70 in the presence of [methyl-^{14}C]thymidine. Centrifugation was for 2.5 h at 40,000 rpm in a Beckman SW41 rotor, using 250 μl of labeled samples that were layered on top of 11-ml 5 to 20% linear sucrose gradients. The same amount of radioactivity was analyzed on all gradients: about 45,000 cpm of [^{3}H]DNA and 20,000 cpm of [^{14}C]DNA. Collected fractions were assayed for trichloroacetic acid-insoluble ^{3}H and ^{14}C counts as previously described (12).*

hydroxymethylase deficiency by DNA polymerase alterations.

Model 1. This model proposes that the T4 43- and 42-proteins do not interact in vivo. The T4 DNA lesions that result from dCMP-hydroxymethylase deficiency in T4 *H4201*-infected *E. coli* B^E may be a reflection of perturbances in the mechanisms that control entry of nucleotide precursors into DNA. The hydroxymethylase appears to be specifically required for utilization of 5-hydroxymethyl-dCTP (27), and a deficiency in this enzyme may lead to limited utilization of dCTP. The incorporation of dCTP, which does not require the T4 gene 42 function (17, 27), may occur to some extent in spite of the presence of the phage-induced dCTPase (gene 56). The presence of cytosine in place of hydroxymethyl cytosine in the phage DNA would make this DNA susceptible to attack by phage-induced cytosine-specific DNases. Gene 43 mutations that suppress the effects of hydroxymethylase deficiencies may either lead to improvement in the fidelity of base selection so that less dCTP is utilized or improve (directly or indirectly) DNA repair activities that counteract nuclease damage. It is possible that some of the T4 *H4201*-pseudorevertants that could not be identified in this study represent mutations in T4 genes that, like gene 43, play roles in the control of the fidelity of phage DNA metabolism.

Model 2. This model proposes that the 43- and 42-proteins interact in vivo. Although extractable dCMP-hydroxymethylase activity did not appear to be specifically modified by DNA polymerase alterations (Table 4), it is still possible that the two proteins undergo transient interactions in vivo or that their interactions do not influence the hydroxymethylase activity of the 42-protein. It is also possible that in T4 *H4201*-infected *E. coli* B^E, the loss or near loss of 42-protein from a multienzyme replication complex may lead to a rearrangement of the components of this complex and to a decrease in the fidelity of DNA copying. Alterations in

other components of the complex, e.g., in the 43-protein, may lead to new rearrangements that improve the fidelity of synthesis. It will be interesting to find out if different lesions in T4 gene 42 are suppressed by different alterations in T4 gene 43 and in other T4 genes. Such would be the case if the products of these genes did interact in vivo. Other more direct approaches at demonstrating the existence of replication complexes are also possible (18a), although some studies (18, 26) suggest that different methods of extraction may have different effects on the functional and structural integrity of the presumed complexes. Recently, Reddy et al. (in press) isolated a complex that contained several T4-induced proteins including dCMP-hydroxymethylase, but no DNA polymerase. This complex appeared to also lack several known DNA-binding proteins that are suspected to play roles in T4 DNA replication, e.g., the 32- and 62-proteins. It is possible that in vivo dCMP-hydroxymethylase participates in more than one type of complex or that some isolation procedures tend to dissociate certain activities from a very large complex of enzymes that controls both DNA precursor biosynthesis and replication fork movement.

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