Isolation and Partial Characterization of Bacteriophage T5 Mutants Deficient in the Ability to Induce Deoxynucleoside Monophosphate Kinase¹

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Two mutants of bacteriophage T5 deficient in the ability to induce wild-type levels of deoxynucleoside monophosphate kinase were isolated and partially characterized. Both mutations were demonstrated to be in a structural gene for the kinase. One of the mutants, designated *dnk* 10, induces no detectable levels of dCMP, dGMP, or dTMP kinase activity. Because the mutant can successfully infect nonpermissive cells, phage-induced deoxynucleoside monophosphate kinase appears to be an unessential function for phage production. DNA synthesis in *dnk* 10-infected cells, however, is reduced to 30% of that observed in wild-type-infected cells; phage production is reduced by a comparable amount. The *dnk* mutation has been mapped and located on the "C" region of the T5 genetic map, 6.3 map units from the C1 locus.

Upon infection of *Escherichia coli* with bacteriophage T5, several early enzymes involved in DNA metabolism are induced, including thymidylate synthetase (2), deoxynucleoside monophosphate kinase (4, 5), dihydrofolate reductase (18, 20), and DNA polymerase (21, 24). Mutants lacking the latter two of these activities have been isolated, and the genetic loci responsible for the two lesions have been mapped (8, 14).

The deoxynucleoside monophosphate kinase induced by T5 has been purified and characterized. The enzyme is tetrafunctional, catalyzing the phosphorylation of dAMP, dCMP, dGMP, and dTMP (5). In contrast, *E. coli* has four separate enzymes, each specific for one of the above four nucleotides (15). The T-even bacteriophages also induce multifunctional kinases; in this case, however, the substrates are dGMP, dTMP, and the unusual nucleotide hydroxymethyldeoxycytidylate which replaces dCMP in the DNA of T-even phages (3, 19). A functionally active kinase has been shown to be required for phage production in T4-infected cells (10), presumably due to the inability of host kinases to phosphorylate hydroxymethyldeoxycytidylate. Because T5 contains the same four deoxynucleotides present in *E. coli* DNA (19), the *E. coli* kinases should be capable of phosphorylating all T5 DNA precursors. Thus we predicted that the T5 kinase should not be an essential

function for successful phage infection.

In this paper we report the isolation of two mutants of T5 deficient in the ability to induce deoxynucleoside monophosphate kinase by using techniques designed to obtain mutants in non-essential genes. These mutants have been partially characterized, and the mutations have been mapped. Both mutations occur in the structural gene for the enzyme.

MATERIALS AND METHODS

Chemicals and equipment. Dihydrofolic acid was prepared from folic acid by the method of Futterman (12). Reduced nicotinamide adenine dinucleotide phosphate was purchased from Sigma Chemical Co. [¹⁴C]thymidylic acid was obtained from either Schwarz BioResearch Inc., or Nuclear Dynamics Inc.; [¹⁴C]thymidine triphosphate, [¹⁴C]thymidine, [¹⁴C]deoxyadenosine triphosphate, [¹⁴C]deoxyguanosine monophosphate, and [¹⁴C]deoxycytidine monophosphate were obtained from Schwarz BioResearch Inc. Polyethyleneimine-impregnated cellulose thin-layer plates (Polygram CEL 300) were purchased from Brinkman Instruments Inc., and prepared according to Randerath and Randerath (23). Radioactivity was measured with either a planchet counting system (Nuclear-Chicago Corp.; model 4338) or a scintillation counter (Beckman; model LS-235). The scintillation fluid used for counting paper disks consisted of 6 g of 2,5-diphenyloxazole per liter of toluene; that for aqueous samples contained 6 g of 2,5-diphenyloxazole per liter of Triton X-100-toluene (1:2).

Growth of bacteria and phage. Nutrient broth and agar were as described by Edgar (11). Minimal

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glucose-salts-maleate (MGM) medium and buffer were prepared according to Lanni (16). All media and plating agar used in these experiments contained 10^{-3} M CaCl_2 . *E. coli* F (su⁻) and *E. coli* F₈ (su⁺), fast-adsorbing strains for T5, which are restrictive and permissive hosts respectively for *amber* mutants, have been described (13). The T5 *amber* mutants used in this study are *am* H18c (B2), *am* H48a (C1), *am* H46b (C2), *am* H51a (D3), *am* H18a (D9), *am* M123c (C0.1), and the double *amber* mutants *am* H59 (C1)·*am* H46b (C2) and *am* M123c (C0.1)·*am* H48a (C1). (The number of the gene containing the mutation is given in parentheses. The most recent map has been published by Hendrickson and McCorquodale [13].) All but *am* M123c and the double *am* M123c·*am* H48a have been previously described (13); *am* M123c was produced by using the same technique that yielded the other listed *amber* mutants. *Am* H48a and *am* H59 presumably contain identical mutations in the C1 gene as *am* H59 is an isolate from a backcross of *am* H48a and T5 *am*⁺. Cultures were grown by gyratory shaking at 30 or 37 C. Chloroform was added to samples before titering. Infective center titers were determined after adsorbing all unadsorbed phage to T5 specific antisera. Phage were preadsorbed to the appropriate host before plating. All plates were incubated overnight at 30 C.

T5 antisera was prepared basically according to Adams (1) against wild-type phage. Phage were purified by two cycles of high- and low-speed centrifugation. A rabbit was injected with these particles as follows: day 1, 2.1×10^{10} particles injected intravenously; day 4, 2.1×10^{10} particles in Freund adjuvant, subcutaneously; day 36, 3.8×10^{11} particles, subcutaneously. The rabbit was bled on day 45, yielding antisera which inactivated T5⁺ with a first order rate constant of 79.2 min^{-1} at 37 C.

Production and screening of mutants. The procedure used to obtain phage mutants in non-essential genes was that of Tessman (25). The phage titer dropped logarithmically to a survival of about 10^{-6} in the sample containing hydroxylamine during a 76-h incubation at 37 C; the titer in a control sample containing no hydroxylamine dropped only to 10% during a similar incubation. Survivors were plated on *E. coli* F from which 101 isolates were selected.

Extracts were prepared from 85 of the above isolates in *E. coli* F according to the procedure described below and assayed for dTMP kinase activity. Those isolates deficient in the ability to induce wild-type levels of kinase were designated *dnk*. One of these, *dnk* 10, was genetically purified by backcrossing it twice to wild-type T5 in *E. coli* F by using wild-type/*dnk* ratios of 1.0 in the first cross and 10 in the second.

Preparation of extracts. To prepare extracts for the isolation, purification, and mapping of *dnk* 10 and 36, 10 ml of a culture of *E. coli* F or F₈ (su⁺) were infected at a multiplicity of infection of 5 to 7.5. After 35 min at 30 C, 5-ml samples were taken, rapidly chilled on ice, centrifuged for 15 min at $10,000 \times g$, and treated with a mixture of lysozyme-EDTA according to a procedure described by Warner et al. (27). When larger and more concentrated extracts

were required, the method of Hendrickson and McCorquodale (14) was employed except that 100-ml samples were withdrawn at the various time points, and, after centrifugation, resuspended in a final volume of 1.5 ml of 0.05 M glycyglycine buffer (pH 7.0) containing 10^{-3} M 2-mercaptoethanol.

Mapping. To determine in which area of the T5 genetic map the gene responsible for the *dnk* mutation is located, *dnk* 10 was crossed with various T5 single *amber* mutants and the non-*amber* progeny were screened for their ability to induce deoxynucleotide kinase. Crosses were performed as described by Hendrickson and McCorquodale (13) with an input ratio of 5 to 7.5 PFU per cell of each mutant. Progeny phage were preadsorbed and plated on su⁺ cells. Non-*amber* progeny, ranging from 25 to 50 per cross, were then used to make extracts in *E. coli* F which were assayed for dTMP kinase activity. Recombinational frequency was calculated as the number of *dnk*⁺ isolates per number of non-*amber* isolates screened for kinase activity.

Three factor crosses between *dnk* 10 and the double *amber* mutants *am* H59·*am* H46b and *am* M123c·*am* H48a were also performed. In these experiments, individual plaques from 1,000 progeny phage were resuspended in 0.50 ml of nutrient broth in autoclavable nylon boxes containing 25 wells. These resuspended plaques were then spot complemented against the individual parental *amber* mutants according to the method of Doerman (9) by using a multiprong applicator. Progeny resulting from crossovers between the two *amber* markers were selected and used to make extracts to assay kinase, as described in the extract preparation section.

Enzyme assays. Deoxynucleoside monophosphate kinase activity was determined as described by Wiberg et al. (28). The reaction mixture used to assay dTMP and dCMP kinase contained in a final volume of 0.50 ml: ATP, 2.0 μmol ; MgCl_2 , 4.0 μmol ; KCl, 40.0 μmol ; Tris buffer, pH 7.0 or 7.5, 50.0 μmol ; deoxynucleoside monophosphate, 0.20 μmol ; ¹⁴C-labeled deoxynucleoside monophosphate (54 to 116 mCi/mmol), 0.04 μCi , and crude extract containing 0.40 to 20.0 units of enzyme. This mixture was incubated at 30 C for 10 min, and the reaction was stopped by the addition of 0.60 ml of cold 0.10 M EDTA, pH 8.1, and placing the mixture on ice. Substrates and products were separated by passing the entire sample through a column (0.50 by 5.0 mm) of Dowex-1-8X-formate ion-exchange resin (200 to 400 mesh) and progressively eluting with 2.0 ml of water, 9.0 ml of 0.25 M ammonium formate, pH 4.3, and 3.0 ml of 2.00 M ammonium formate, pH 4.3, for thymine-containing nucleotides, and with 2.0 ml of water, 6.0 ml of 0.06 M ammonium formate, pH 2.65, and 3.0 ml of 1.0 M ammonium formate, pH 5.0, for cytosine-containing nucleotides. In both cases, all diphosphates and triphosphates are co-eluted in the last wash, samples of which were counted either in Triton X-100-toluene or on planchets. dGMP kinase activity was determined by using the same assay except on a 10-fold smaller scale and the exception of radioactively labeled substrate which was maintained at the above level. The reaction was terminated by placement in a

boiling water bath for 1 min. A 20- μ liter sample was applied to a polyethyleneimine-impregnated plate along with standards, washed in 50% methanol for 15 min, and chromatographed in 1 M LiCl according to the procedure of Randerath and Randerath (23). After UV visualization, mono-, di-, and triphosphates were cut out and counted directly in toluene-2,5-diphenyloxazole. Under these conditions dGDP and dGTP migrate as one elongated spot near the origin. In both assay procedures 1 unit of kinase is defined as the amount required to catalyze the formation of 1 nmol of product under these conditions.

DNA polymerase assays were performed by measuring the conversion of acid-soluble radioactive deoxynucleoside triphosphates into acid-insoluble material according to the filter paper disk method designed by Bollum (6). The reaction mixture employed was identical to that described by Steuart et al. (24) except that it also contained 1.0 mM ATP. The salmon sperm DNA used as primer was heat denatured at 100 C for 30 s and rapidly cooled in ice. One unit of polymerase is defined as that amount which converts 1.0 nmol of labeled deoxynucleoside triphosphate to acid-precipitable material in 30 min at 37 C.

Dihydrofolate reductase activity was determined by the spectrophotometric assay described by Warner and Lewis (26). One unit of reductase activity is defined as producing a decrease of 0.01 in optical density at 340 nm in 1 min at room temperature under standard assay conditions.

DNA analyses. Bacteria were grown, concentrated, and infected as described previously (14). MGM minimal media and buffer were used instead of M9. Analysis of the total DNA present after infection by the Dische diphenylamine reaction as modified by Burton has also been described (14) except that phage growth was initiated by diluting the culture 10-fold into prewarmed (37 C) nutrient broth and commencing aeration. For the determination of incorporation of [14 C]thymidine into DNA, phage growth was initiated by diluting the phage-bacterial complexes 10-fold into nutrient broth containing 500 μ g of uridine per ml and 0.05 μ Ci of [14 C]thymidine per ml (specific activity, 50 μ Ci/ μ mol) and aerating. At various times 1.0-ml samples were removed and added to 1.0 ml of cold 10% trichloroacetic acid. After 30 min at 0 C, the precipitates were sedimented by centrifugation for 15 min at 2,500 $\times$ g, washed twice with 2 ml of cold 5% trichloroacetic acid, and dissolved in 0.50 ml of 1 M NH_4OH . Samples plus 0.8 ml of water were counted in Triton X-100-toluene. The method employed for the determination of the rate of incorporation of [14 C]thymidine into DNA was that described by Hendrickson and McCorquodale (14) except that the pulse mixture contained 500 μ g of uridine and 0.20 μ Ci of [14 C]thymidine (specific activity, 50 μ Ci/ μ mol) in 0.10 ml of nutrient broth. After a 2-min pulse at 37 C, 1.0 ml of 10% trichloroacetic acid was added to the phage-bacteria complexes. The amount of radioactivity present in the precipitates was determined in the same manner as that described above for cumulative incorporation of [14 C]thymidine into DNA.

RESULTS

Identification of a deoxynucleoside mono-

phosphate kinase-deficient mutant. Of 85 isolates from heavily mutagenized phage stocks, two failed to induce the usual increase in kinase activity after infection. These have been designated *dnk* for their apparent lack of kinase activity. One of these was backcrossed twice to wild-type T5 in an attempt to separate any secondary mutations present. The resulting mutant, *dnk* 10, produces no detectable increase in kinase activity after infection at 30 C when measured with dCMP, dGMP, or dTMP as substrate (Fig. 1). This mutation is not suppressed by the *amber* suppressor *E. coli* F $_{\beta}$. Assays for T5-induced dAMP kinase activity in crude extracts were unreliable because of the extremely high levels of host enzyme (5). *Dnk* 10-infected cells, however, do induce both T5 DNA polymerase and dihydrofolate reductase (Fig. 2). Because the kinetics of appearance and extent of induction of both these enzymes are comparable to that observed in a wild-type infection, the lesion in *dnk* 10 appears to be in a gene-controlling kinase activity and not in a more general early control gene.

To further establish that the mutation responsible for the *dnk* phenotype is in the structural gene for the kinase, use was made of the second mutant isolated unable to induce normal levels of kinase activity. This mutant, designated *dnk* 36, produces a level of kinase activity upon infection at 30 C that is approximately 35% of that observed in T5 $^{+}$ -infected cells. This inducible activity is demonstrably more heat-sensitive than is the wild-type activity (Fig. 3). At 40 C the mutant enzyme loses activity both more rapidly and to a greater extent than the wild-type enzyme. Thus, it would appear that the mutation in *dnk* 36 results in the production of a more heat-labile protein, and is therefore in the structural gene for the kinase.

Because *dnk* 36 would appear to be a muta-

FIG. 1. Lack of induction of deoxynucleoside monophosphate kinase activity in cells infected with T5 $^{+}$ (●) and *dnk* 10 (○) when assayed with dTMP, dGMP, and dCMP. Specific activity represents units per micrograms of protein.

FIG. 2. Induction of dihydrofolate reductase in $T5^+$ (●) and $dnk 10$ (○) infected cells. Although DNA polymerase activity is somewhat lower in $dnk 10$ -infected cells (□) than in $T5^+$ -infected cells (■), it is still substantial. Specific activity represents units per micrograms of protein.

tion in a structural gene for the kinase, experiments were performed to indicate if the $dnk 10$ lesion were in the same gene and therefore also a structural gene mutation. A cross was performed between $dnk 10$ and $dnk 36$, and 50 progeny were screened for their ability to induce wild-type levels of kinase activity. The results shown in Table 1 indicate that only one isolate, or 2% of the progeny, induces kinase levels comparable to those seen in a wild-type infection and thus was the product of a recombinational event between $dnk 10$ and 36. Although few data are available as to the frequency of intragenic recombination observable in $T5$ genetic crosses, 2% is well within the range seen with at least two $T5$ genes (17) and various $T4$ genes (20). Thus, the $dnk 10$ and 36 mutations probably are both located in the structural gene for the kinase.

Effect of the dnk mutation. The pattern of DNA metabolism observed in $dnk 10$ -infected cells is shown in Fig. 4. Synthesis appears to begin at the same time as in $T5$ wild-type-infected cells but proceeds at a slower rate; the starting level of DNA is reached after 45 min at 37 C compared with 26 min for $T5^+$. This pattern has previously been observed in (su^-) cells infected with various *amber* mutants of $T5$ and been designated DR or DNA reduced (14).

That the rate of DNA synthesis is lower in $dnk 10$ infections is confirmed by the pulse labeling experiments shown in Fig. 5. The total amount of DNA synthesized, as measured by incorporation of radioactively labeled thymidine into acid-precipitable material, is also reduced in mutant-infected cells (Fig. 6). Total accumulation amounts to 32% of that observed in a comparable $T5^+$ infection. Similarly, phage burst size is reduced to 33% of that observed for wild type.

Mapping of the dnk mutation. Although the mutation in $dnk 10$ reduces phage burst size, the plaque size of $dnk 10$ plated under a variety of conditions did not differ significantly from that of $T5^+$. Genetic mapping was therefore accomplished by performing kinase assays on extracts prepared from individual progeny of a cross. Two-factor crosses were performed be-

FIG. 3. Heat lability of the deoxynucleoside monophosphate kinase activity induced by $dnk 36$ (○) compared to the enzyme activity induced by $T5^+$ (●). The left ordinate expresses $dnk 36$ and $T5^+$ activity as a fraction of $dnk 36$ and $T5^+$ initial activity, respectively. The right ordinate expresses $dnk 36$ activity as a fraction of $T5^+$ initial activity. Individual points represent the time of incubation of a crude extract at 40 C. Preincubation was halted, and the enzyme reaction was begun by adding 0.10 ml of the preincubation mix to 0.40 ml of ice-cold standard kinase reaction mix, which was then placed at 30 C and incubated for 12 min. The assay substrate was dTMP. All other experimental conditions are described in Materials and Methods.

TABLE 1. Recombination between $dnk 10$ and $dnk 36$

Type of progeny	Mean sp act	No. of progeny	Percentage
Total		50	100
$dnk 36$	0.338 ± 0.108	26	52
$dnk 10$	0.017 ± 0.006	23	46
$T5^+$	1.000 ± 0.149	1	2

FIG. 4. DNA metabolism after infection with *dnk* 10 (O) and *T5*⁺ (●). The amount of total DNA present in infected cells at 37°C was determined chemically by the Dische diphenylamine reaction as described in Materials and Methods.

FIG. 5. Rate of DNA synthesis after infection with *dnk* 10 (O) and *T5*⁺ (●). Individual points are plotted as a function of the time at which samples were taken to begin a 2.0-min pulse at 37°C with [¹⁴C]thymidine and represent the total amount of radioactivity incorporated into acid-precipitable material during the 2-min incubation. All other experimental procedures are described in Materials and Methods.

tween *dnk* 10 and four *amber* mutants located in three of the four regions of the T5 genetic map; *am* H18c (B2), *am* H48a (C1), *am* H51a (D3), and *am* H18a (D9). Although the segments A, B, C, and D are no longer considered

to be genetically independent regions of the T5 genome (A. H. Doermann, personal communication), crosses between *dnk* and representatives from each area should roughly place *dnk* on the map. The results from these crosses indicate that the *dnk* lesion is located in the "C area" near gene C1 (Table 2).

To determine the location of *dnk* more precisely, three-factor crosses were performed between *dnk* 10 and the double *amber* mutants *am* H59 (C1)·*am* H46b (C2), and *am* M123c (C0.1)·*am* H48a (C1). The recombination frequencies between *dnk* 10 and *am* H48a (C1), between *am* M123c (C0.1) and *am* H48a (C1), and between *am* H59 (C1) and *am* H46b (C2), are 0.065 (Table 2), 0.088 (based on 10 crosses yielding a standard deviation of ± 0.007), and 0.125 (13), respectively. Because *dnk* 10 maps closer to C1 than does either C0.1 or C2, the *dnk* gene should either lie between C0.1 and C1 or between C1 and C2. By using the recombination frequencies it is possible to predict the nature of the progeny expected from each cross depending upon whether *dnk* lies within or outside of the interval between the two *am* markers used in

FIG. 6. Incorporation of [¹⁴C]thymidine into acid-precipitable material after infection at 37°C with *dnk* 10 (O) and *T5*⁺ (●) compared with PFU per milliliter from infection with *dnk* 10 (O) and *T5*⁺ (●). Phage yields are plotted as PFU per infected bacteria, and hence the maximal value represents approximate burst size. Phage were titrated after chloroform treatment of infected cells and thus represent intracellular phage pools. Other experimental procedures are described in Materials and Methods.

TABLE 2. Mapping of *dnk* 10 by two factor crosses

Cross	No. of <i>am</i> ⁺ progeny screened	No. of <i>dnk</i> progeny	Recombination frequency
<i>am</i> H18c (B2) × <i>dnk</i> 10	50	15	0.300
<i>am</i> H48a (C1) × <i>dnk</i> 10	46	3	0.065
<i>am</i> H51a (D3) × <i>dnk</i> 10	58	15	0.256
<i>am</i> H18a (D9) × <i>dnk</i> 10	25	11	0.440

each cross. The results shown in Table 3, based on 32 recombinations between C0.1 and C1 and 31 recombinations between C1 and C2, indicate that *dnk* 10 lies between genes C0.1 and C1 rather than between C1 and C2. The data obtained from the cross between *dnk* 10 and *am* M123c-*am* H48a can be used to calculate the location of *dnk* 10 more precisely than the data given in Table 2; this value is $0.72 \times 0.088 = 0.063$. The mapping data for the C region of the T5 genome already available (13) and that obtained for this paper are summarized in Figure 7. After nomenclature soon to be published (D. J. McCorquodale, manuscript in preparation), the *dnk* locus would be designated as gene C0.3.

DISCUSSION

Three lines of evidence indicate that *dnk* 10 and *dnk* 36 carry mutations in the structural gene coding for T5 deoxynucleoside monophosphate kinase: (i) *dnk* 10 and 36 both fail to induce normal levels of deoxynucleoside monophosphate kinase and the two mutations recombine with a frequency of only 2%, (ii) the kinase induced by *dnk* 36 is more heat labile than that induced by wild-type T5, and (iii) *dnk* 10 induces no kinase activity, but induces about 75 to 80% of the normal levels of dihydrofolate reductase and DNA polymerase, indicating that the mutation is not in a regulatory gene for early enzyme synthesis.

Figure 7 summarizes the results of the mapping of *dnk* 10. The data are consistent with the placement of this gene between genes C0.1 and C1. The data in Table 3 would indicate that it is very unlikely that *dnk* 10 lies outside but adjacent to this region, although it provides only a rough approximation of the exact location of *dnk* 10 within the region. This is the first report of an early enzyme mapping within the C-region of the T5 genetic map, although the

gene products of genes C0.1 and C1 have not yet been characterized. The product of the C2 gene is a sigma-like factor required for normal turn-on of synthesis of late RNAs and proteins (7). It is unlikely that the 20% reduction of dihydrofolate reductase and DNA polymerase activity induced by *dnk* 10 is due to polar effects because these enzymes map in genes B3 and D9, at considerable distances from *dnk*. The reason for the small but significant reduction in the amount of dihydrofolate reductase and DNA polymerase induced is not known.

Because *dnk* 10 can successfully infect *E. coli*, the presence of a functionally active phage-specific deoxynucleoside monophosphate kinase would not appear to be essential for some phage production. However, both DNA yield and burst size are reduced to approximately 30% of that observed in comparable wild-type infections. A similar effect has been observed with T5 and T4 mutants deficient in the ability to induce dihydrofolate reductase (14, 19) and with other early T4 genes (19) involved in nucleotide metabolism whose function appears to be enhancement of already present host functions thus permitting maximum phage production but whose presence is not required for

FIG. 7. Location of *dnk* 10 on a segment of the T5 genetic map. The map unit distances given between *dnk* 10 and genes C0.1 (*am* M123c) and C1 (*am* H48a) are from the three-factor crosses described in Table 3. The recombinational frequency between genes C1 and C2 has been previously reported (13); that between C0.1 and C1 was determined as described in Materials and Methods. Nomenclature is that suggested by D. J. McCorquodale (manuscript in preparation).

TABLE 3. Mapping of *dnk* 10 by three factor crosses

Cross	Progeny screened					Predicted <i>dnk</i> ⁻ (%)	
	<i>am</i> Genotype	Total screened	<i>dnk</i> ⁺	<i>dnk</i> ⁻	% <i>dnk</i> ⁻	If <i>dnk</i> ^a inside	If <i>dnk</i> ^b outside
<i>am</i> M123c (C0.1)- <i>am</i> H48a (C1) × <i>dnk</i> 10	C0.1 ⁺ C1 ⁻	32	9	23	72	74	6.5
<i>am</i> H59(C1)- <i>am</i> H46b (C2) × <i>dnk</i> 10	C1 ⁻ C2 ⁺	31	29	2	6.9	52	6.5

^a Calculated by dividing 0.064 by 0.088 and 0.125, respectively.

^b Recombination frequency between *dnk* and *am* H48a from Table 2.

some minimal level of phage growth. We cannot unequivocally conclude that the host dNMP kinases are completely responsible for the limited DNA synthesis that is observed in cells infected with *dnk* 10 because we have no evidence to rule out the possibility that the defective phage-induced enzyme retains some *in vivo* activity.

The absence of the phage T5-induced dNMP kinase is not lethal as is the absence of the phage T4-induced dNMP kinase. The difference is probably due to the absence of hydroxymethyl-dCMP kinase activity in uninfected *E. coli* so that one of the precursors for T4 DNA synthesis cannot be synthesized, whereas all of the precursors for T5 DNA synthesis can presumably be synthesized without the phage-induced enzyme. However, the lower rate of DNA synthesis when the T5-induced kinase is absent suggests that deoxyribonucleoside triphosphate pools may be too low for optimal DNA synthesis. An alternative explanation is that the kinase may be part of a DNA-synthesizing complex as has been suggested for T4 (29), and in its absence DNA synthesis cannot proceed at wild-type levels because the complex is missing one of its components. Although *dnk* 10 has been backcrossed twice with wild-type T5, it is still possible that at least one other mutation is present which affects DNA synthesis in cells infected with this mutant, and that the defects in kinase activity and DNA synthesis are unrelated.

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