Gene *1.7* of bacteriophage T7 confers sensitivity of phage growth to dideoxythymidine

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Bacteriophage T7 DNA polymerase efficiently incorporates dideoxynucleotides into DNA, resulting in chain termination. Dideoxythymidine (ddT) present in the medium at levels not toxic to *Escherichia coli* inhibits phage T7. We isolated 95 T7 phage mutants that were resistant to ddT. All contained a mutation in T7 gene *1.7*, a nonessential gene of unknown function. When gene *1.7* was expressed from a plasmid, T7 phage resistant to ddT still arose; analysis of 36 of these mutants revealed that all had a single mutation in gene *5*, which encodes T7 DNA polymerase. This mutation changes tyrosine-526 to phenylalanine, which is known to increase dramatically the ability of T7 DNA polymerase to discriminate against dideoxynucleotides. DNA synthesis in cells infected with wild-type T7 phage was inhibited by ddT, suggesting that it resulted in chain termination of DNA synthesis in the presence of gene 1.7 protein. Overexpression of gene *1.7* from a plasmid rendered *E. coli* cells sensitive to ddT, indicating that no other T7 proteins are required to confer sensitivity to ddT.

deoxynucleotide kinase | dideoxynucleosides | DNA metabolism | T7 DNA polymerase | thymidine kinase

When bacteriophage T7 infects *Escherichia coli*, the host RNA polymerase transcribes T7 early genes, designated class I genes. This group includes the T7 RNA polymerase (gene *1*) (1). Approximately 6–15 min after infection, T7 RNA polymerase transcribes a second group of T7 genes, designated class II genes, that encode most of the proteins involved in DNA metabolism. The class II genes include those that encode the helicase/primase (gene *4*), the DNA polymerase (gene *5*) (1), and a single-stranded DNA-binding protein (gene *2.5*) (2). A number of class II genes are not essential under laboratory growth conditions and have not yet been assigned specific functions (1).

Little is known about the process of deoxynucleotide metabolism in T7-infected *E. coli*. T7 derives most of the phosphorus incorporated into its DNA from the breakdown of *E. coli* DNA (3, 4). The host DNA is degraded to 5′-deoxynucleoside monophosphates (dNMPs) by the joint action of the gene *3* endonuclease (5) and gene *6* exonuclease (6). It is not known whether the phage encodes its own enzymes to convert dNMPs to the dNTP precursors for DNA synthesis. *E. coli* encodes four different dNMP kinases, each specific for one dNMP (7, 8). At least one of these kinases, CMP kinase, encoded by *cmk*, is essential for T7 growth (9). In turn, nucleoside diphosphokinase is the enzyme primarily responsible for conversion of dNDPs to dNTPs (10). Phage T4 encodes its own set of dNMP kinases with specificities that differ from that of the *E. coli* enzymes, whereas it uses the *E. coli* nucleoside diphosphokinase to convert the dNDPs to dNTPs (11). Phage T4 also encodes a thymidine kinase (*tdk*) (12, 13). Neither *E. coli* nor phage T7 will readily use exogenous thymine for DNA synthesis; in contrast, phage T4 efficiently incorporates exogenous thymine into its DNA (13).

One of the interesting properties of T7 DNA polymerase is its ability to incorporate dideoxynucleoside monophosphates (ddNMPs) more efficiently than most DNA polymerases, including the homologous *E. coli* DNA polymerase I (14) and *Thermus aquaticus* DNA polymerase (15). This increased ability is due to a single amino acid residue, tyrosine-526, in T7 DNA polymerase; both *E. coli* DNA polymerase I and *Taq* polymerase have a phenylalanine at the equivalent position (15). The crystal structure of a T7 DNA polymerase–dNTP complex shows the hydroxyl group of the tyrosine interacting with the β-phosphate of the ddNTP, presumably stabilizing it (16). Incorporation of a ddNMP results in chain termination, halting DNA synthesis. Consequently, we tested whether T7 phage may be sensitive to dideoxynucleosides in the medium at concentrations that would not affect the growth of *E. coli*. We have identified conditions whereby T7 phage growth is inhibited by dideoxythymidine (ddT) at concentrations that do not inhibit *E. coli* growth. We have isolated T7 phage that are resistant to ddT and characterized the nature of these mutations. The most frequent mutations occur in T7 gene *1.7*, a nonessential gene of unknown function that lies within the class II cluster of genes whose products are involved in T7 DNA metabolism.

Results

Sensitivity of T7 Phage to Dideoxynucleosides. T7 DNA polymerase is one of the few DNA polymerases that incorporate ddNMPs with essentially the same efficiency as it does dNMPs (15). In contrast, the host *E. coli* DNA polymerase III, the replicative polymerase, and DNA polymerase I, the major repair enzyme, discriminate against ddNMPs several hundredfold (15, 17). We were interested to see whether T7 phage would be inhibited by these nucleoside analogs at a concentration not toxic to the host. We analyzed the plating efficiency of T7 phage in the presence of varying concentrations of ddA, ddT, and ddC (Fig. 1). We did not examine growth in the presence of ddG because of its limited solubility. It has been shown that relatively high concentrations of ddA inhibit DNA synthesis in *E. coli* and lead to cell death whereas ddC has no effect on cell growth (18).

The presence of ddC up to 2.5 mM had essentially no effect on the plating efficiency of phage T7. The presence of 2.5 mM ddA reduced the plating efficiency of phage T7 by 3-fold, but at this concentration the host cells also grew poorly. We cannot explain why we actually observe stimulation of the plating efficiency of T7 in the presence of low concentrations of ddA (0.1 mM) or high concentrations of ddC (>0.7 mM). In contrast, low concentrations of ddT had very little effect on the growth of *E. coli* but dramatically reduced the plating efficiency of T7 phage; the presence of 1 mM ddT reduced the plating efficiency of phage T7 by a factor of 1,000.

Isolation of Phage T7 Mutants Resistant to ddT. Although concentrations of ddT >0.5 mM dramatically decreased the plating

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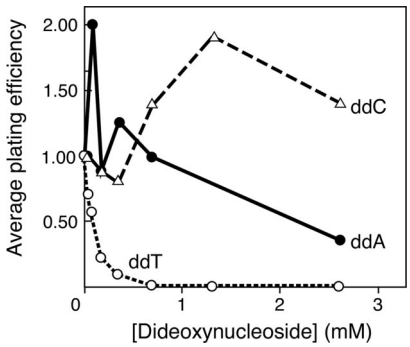

Fig. 1. Dideoxythymidine (ddT) strongly inhibits T7 phage. *E. coli* HMS89 was infected with WT T7 phage and grown in Davis minimal medium supplemented with increasing concentrations of ddT (○), ddA (●), or ddC (△). Plating efficiencies were calculated by dividing the number of plaques produced in the presence of the indicated dideoxynucleoside by the number of phage produced in its absence. Results are the average of three experiments.

efficiency of T7 phage, approximately 1 in 1,000 phage produced plaques of normal size. When phage from these plaques were titered on *E. coli* HMS89, the presence of ddT did not affect their efficiency of plating or plaque size. Thus, mutations arise at a high frequency in T7 which renders the phage insensitive to the presence of ddT. Mutant phage were isolated for further characterization.

Identification of the T7 Locus Responsible for ddT Sensitivity by Deletion Mapping. Physical mapping of one of the mutations that render T7 resistant to ddT revealed that it was upstream of nucleotide 9490 (data not shown). This region encodes class I and class II genes of T7 (19). Many of these genes are not essential for T7 growth, and a number of deletions in this region have been mapped (20). Analysis of a series of these deletion mutants for their ability to grow in the presence of ddT suggested that at least one class of mutants resistant to ddT is due to inactivation of a gene that lies between nucleotide 8442 and 8660 from the left end of the T7 genome (Fig. 2). This region lies in the middle of gene *1.7*, a gene of unknown function. To define this region further, we studied a deletion mutation between two *Sau3A*I sites, between nucleotides 8312 and 8415 (Δ1.7-1). This

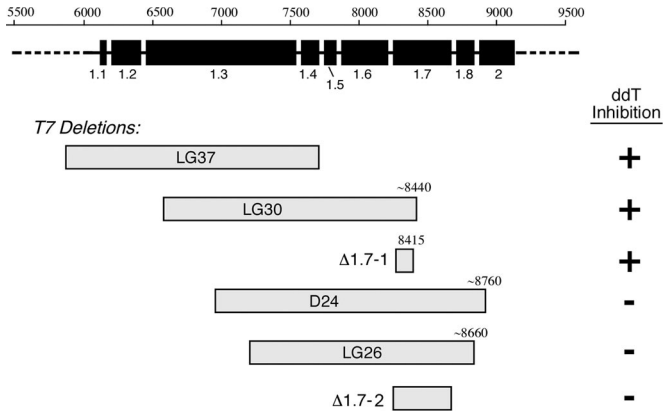

Fig. 2. Deletion mapping of the T7 region responsible for ddT sensitivity. The darkened rectangles depict the regions of T7 encoding gene *1.1* through gene *2*. The nucleotide positions on the T7 genome are denoted above, and the gene number below. A series of deletion phage were used to map the region responsible for ddT sensitivity. The regions of the genome missing from the phage are denoted by the gray rectangles, and the sensitivity of the phage to ddT is indicated in the column at the right. T7Δ1.7-2 is the phage whose entire gene *1.7* is replaced by the *trxA* gene.

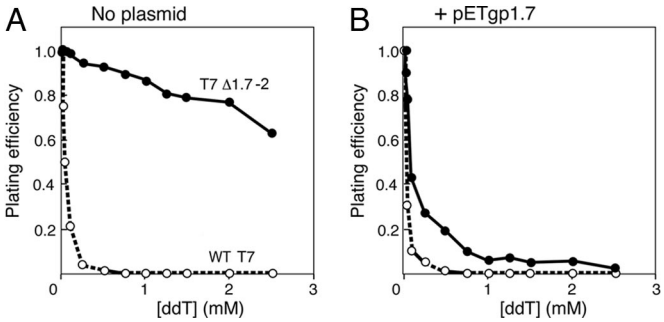

Fig. 3. Gene *1.7* expressed on a plasmid restores sensitivity to ddT. Wild-type (○) and T7 Δ1.7-2 (●) phage were used to infect *E. coli* HMS89 in LB medium supplemented with the indicated concentrations of ddT. Plating efficiencies were calculated as described in Fig. 1. (*A*) *E. coli* HMS89. (*B*) *E. coli* HMS89/pETgp1.7.

103 nucleotide-deleted sequence lies within the NH_2 terminus of the gene *1.7* product. Surprisingly, this deletion mutant was still sensitive to ddT, suggesting that the region critical for ddT resistance lies within the C-terminal half of gene *1.7*. The C-terminal region of the resulting fusion protein is not in-frame with the original start codon for gene *1.7*, and thus in theory, it should not be produced. However, the sensitivity to ddT of T7 phage containing deletion LG30 supports the essential nature of the C terminus of gp1.7 in conferring sensitivity to ddT; the LG30 deletion deletes 92 residues of the NH_2 terminus of gp1.7. One possibility is that the start codon at nucleotide 8446 was used in this deletion mutant to produce a protein consisting of the 103 C-terminal residues of gp1.7. To make further studies unambiguous, we constructed phage T7Δ1.7-2, in which the entire gene *1.7* has been deleted. This mutant phage is resistant to high concentrations of ddT.

Ability of Cloned Gene 1.7 to Restore Sensitivity to ddT. To show definitively that gp1.7 is responsible for conferring sensitivity to ddT, we cloned gene *1.7* and examined whether its expression from a plasmid would render T7 phage T7Δ1.7-2 sensitive to ddT. We constructed the plasmid pETgp1.7 encoding gene *1.7* under the control of a T7 RNA polymerase promoter; cells containing this plasmid will produce gp1.7 when phage T7 infects the cell. When T7Δ1.7-2 infects *E. coli* cells that do not express gene *1.7*, phage growth was resistant to high concentrations (2.5 mM) of ddT (Fig. 3*A*). In contrast, WT T7 phage could not grow even with 0.1 mM ddT. When either T7Δ1.7-2 phage or T7 wild-type (WT) phage infected *E. coli* cells containing the plasmid pETgp1.7, in both cases phage growth was sensitive to 0.1 M ddT (Fig. 3*B*). We consistently observed that WT T7 phage were more sensitive to ddT than were T7Δ1.7-2 phage when grown in cells expressing gene *1.7* from a plasmid. Perhaps the level of gp1.7 or its temporal expression may be important in its function.

We used this complementation assay with pETgp1.7 to examine 95 ddT-resistant phage chosen at random from the pool of ddT-resistant mutant phage described in the previous section. Because these mutants were derived from the same phage pool, we cannot rule out the possibility that some of these mutants were siblings. All 95 phage were inhibited by low concentrations of ddT when they infected cells containing pETgp1.7. We conclude from this observation that a defective gene *1.7* was responsible for the ddT-resistant phenotype in all 95 mutant phage.

DNA Sequence Analysis of Five Mutant T7 Phage Resistant to ddT. We determined the sequence of gene *1.7* in five of the mutant phage that were resistant to ddT. All five had mutations in gene *1.7*; one

Table 1. Mutations in gene *1.7* of phage resistant to ddT

Mutation*	Nucleotide†	Codon	Change in gp1.7
Frameshift‡	+C nt 8401		Premature stop at aa 83
Nonsense	C > A nt 8548	TA**C** > TA**A**	Y128 *term*
Missense	T > C nt 8610	T**T**C > T**C**C	F149S
Missense	T > G nt 8580	G**T**G > G**G**G	V139G
Nonsense	C > A nt 8667	T**C**A > T**A**A	S168 *term*

*Mutations observed in the gene *1.7* region of each of five T7 mutant phage that were complemented by plasmid pETgp1.7 to restore sensitivity to ddT.
†Nucleotide (nt) positions are from the left end of T7, where the first base of gene 1.7 is 8166.
‡This phage is referred to as T7 ddT^{R}1 in this paper.

a frameshift, two were missense, and two were nonsense mutations (Table 1). The frameshift mutant, T7 ddT^{R}1, resulted in a stop codon at amino acid residue 83. Presumably this mutant, in contrast to the deletion mutants described previously, was not able to produce the C-terminal fragment of gp1.7 that can render T7 sensitive to ddT.

Effect of ddT on Phage T7 DNA Synthesis. To determine whether T7 DNA synthesis is affected by the presence of ddT, we measured the incorporation of [^{3}H]thymidine into DNA after infection of *E. coli* (Fig. 4). Immediately after infection of *E. coli* with WT T7 in the absence of ddT, there is a reduction in DNA synthesis as a result of inhibition of host DNA replication (21, 22). Then DNA synthesis increases between 5 and 15 min as T7 DNA replication commences (Fig. 4*A*). In the presence of ddT, there was no DNA synthesis after infection of *E. coli* with WT T7. In contrast, with the ddT-resistant mutant T7 ddT^{R}1, DNA synthesis was inhibited by ddT to a much lesser extent; the overall temporal pattern of increased DNA synthesis 5–15 min after T7 infection was the same as that observed in the absence of ddT (Fig. 4*B*). With both WT and T7 ddT^{R}1 phage, the initial [^{3}H]thymidine incorporated (0–5 min) after infection was significantly reduced by the presence of ddT, presumably because ddT was competing with [^{3}H]dT for uptake into the cell or for phosphorylation by a host enzyme (the ratio of ddT to [^{3}H]dT was 200:1). Finally, when T7 ddT^{R}1 phage infected cells contained the pETgp1.7 plasmid, DNA synthesis was again inhibited by ddT (data not shown). These results show clearly that the inhibition of T7 growth by ddT arises from a cessation of DNA synthesis, presumably because of incorporation of ddTMP into DNA.

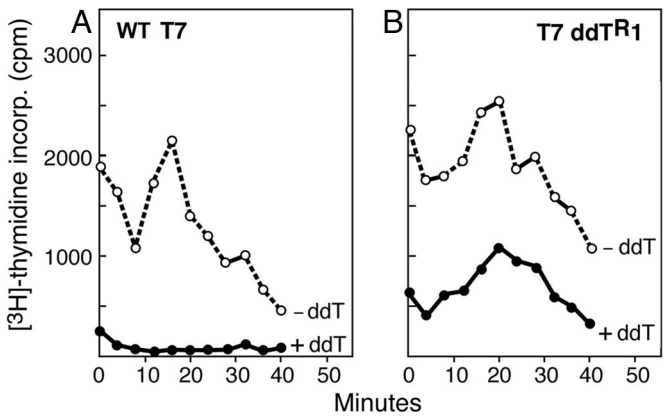

Fig. 4. Effect of ddT on *in vivo* DNA synthesis. DNA synthesis was monitored by the incorporation of [^{3}H]thymidine into phage DNA. WT T7 (*A*) or T7 ddT^{R}1 (*B*) was used to infect *E. coli* in the presence (●) or absence (○) of 0.45 mM ddT.

A Single Mutation in T7 Gene 5 Bypasses Functional gp1.7. The *in vivo* data suggest that ddT inhibits T7 phage growth via its conversion to ddTTP that is incorporated into T7 DNA by T7 DNA polymerase, resulting in chain termination. If so, then a mutation in T7 DNA polymerase that decreases its ability to use ddTTP would bypass gp1.7. We screened for T7 phage that were resistant to ddT because of mutations in genes other than in gene *1.7*, with the expectation that some would reside within T7 gene *5*, the structural gene for the DNA polymerase.

E. coli HMS89 carrying plasmid pETgp1.7 was used for infection of WT T7 in the presence of 1 mM ddT. When WT T7 phage infects this host, gp1.7 is induced both from the phage genome and the plasmid. Thus, T7 phage that would be resistant to ddT because of a mutation in gene *1.7* on the phage will not be able to grow because gp1.7 will still be produced by the gene on the plasmid. Phage resistant to ddT arose when T7 infected *E. coli* HMS89/pETgp1.7 (Table 2). However, the frequency of mutant phage that arose in the presence of pETgp1.7 was reduced by a factor of 100,000-fold compared with that obtained in the absence of pETgp1.7.

A total of 36 ddT mutant phage were picked for characterization. All gave large plaques when grown in *E. coli* HMS89/pETgp1.7 in the presence of 1 mM ddT. None of the phage grew on *E. coli* HMS89/pGP5-3 in the presence of 1 mM ddT. Because this host overproduces WT T7 DNA polymerase, this result suggests that the phage resistant to ddT harbored a mutation in gene *5*. To identify the mutations, we randomly picked nine ddT-resistant phage and sequenced their gene *5*. All had a single mutation in gene *5* in which the adenosine at nucleotide 1577 had changed to thymidine, resulting in the replacement of tyrosine-526 with phenylalanine. We have shown previously that whereas the WT T7 DNA polymerase discriminates 3-fold against the incorporation of ddTTP compared with dTTP, replacement of tyrosine-526 with phenylalanine results in a DNA polymerase that discriminates >8,000-fold (15). Thus, T7 phage encoding a DNA polymerase that discriminates strongly against the incorporation of ddNMPs is not inhibited by high levels of ddT. This resistance to ddT most likely reflects the inability of ddTTP arising from ddT to compete with the normal nucleotide, dTTP.

Gp1.7 Renders *E. coli* Sensitive to ddT. The relative resistance of *E. coli* strains to dideoxynucleosides (18) could arise via several mechanisms. For example, ddT could have limited uptake by the cell, be a poor substrate for the appropriate nucleoside kinase, or not be used efficiently by the *E. coli* DNA polymerases. As mentioned earlier, both *E. coli* DNA polymerases I and III are known to use ddNTPs inefficiently (15, 17).

Because gp1.7 confers sensitivity of T7 phage growth to ddT, we determined whether it would similarly affect *E. coli* growth or, alternatively, if the phenotype was unique to T7, would suggest the possibility that other T7-encoded protein(s) may be involved. λDE3 prophage was inserted into *E. coli* HMS89, allowing the T7 RNA polymerase to be induced by IPTG and leading to overexpression of gene *1.7* harbored on the plasmid pETgp1.7. As anticipated, *E. coli* HMS89(DE3) is not inhibited by 1 mM ddT in the absence of gene *1.7* (Fig. 5*A*). However, *E. coli* HMS89(DE3)/pETgp1.7 is strongly inhibited by 1 mM ddT (Fig. 5*B*). Analysis in the presence of increasing concentrations of ddT showed that the growth of *E. coli* HMS89(DE3)/pETgp1.7 was inhibited 50% by 0.05 mM ddT. In contrast, *E. coli* HMS89(DE3) in the absence of gp1.7 was not inhibited significantly by the presence of even 7.5 mM ddT. Thus, the K_i for ddT in the absence of gp1.7 was >100-fold higher than in the presence of gp1.7 (data not shown).

The data show that overexpression of gp1.7 renders *E. coli* sensitive to ddT. As in the case for T7 infected cells, DNA synthesis in *E. coli* cells expressing gene *1.7* was also inhibited by ddT (data not shown). Presumably the presence of gp1.7 signif-

Table 2. Isolation of ddT-resistant T7 phage with mutations in gene *5*

	WT T7		T7 Δ 1.7–2	
E. coli	Pfu/ml	EOP	Pfu/ml	EOP
HMS89(DE3) − ddT	1.5×10^{10}	1	1.2×10^{10}	1
HMS89(DE3) + ddT	8×10^{6}	5.3×10^{-4}	1.08×10^{10}	0.9
HMS89/pETgp1.7 − ddT	1.5×10^{10}	1	1×10^{10}	1
HMS89/pETgp1.7 + ddT	1.2×10^{2}	8×10^{-9}	7.3×10^{7}	7.3×10^{-3}

E. coli HMS89 and HMS89/pETgp1.7 were infected by either WT or Δ 1.7-2 T7 phage in the presence or absence of 1 mM ddT. Plaques were counted, and the efficiency of plating (EOP) was determined as described in *Materials and Methods*.

icantly increases the pool of ddTTP such that it can successfully compete with dTTP for incorporation into *E. coli* DNA. This result shows that gp1.7 does not require other T7 proteins to confer sensitivity to ddT.

Host Thymidine Kinase Is Essential for gp1.7-Dependent ddT Sensitivity of both T7 Phage and *E. coli*. In *E. coli* the "thymine salvage pathway" is the only way that exogenous thymidine can be converted to dTTP (23). This pathway requires tdk to convert thymidine to thymidylate (dTMP) (24). *E. coli* defective in tdk are unable to incorporate exogenous thymidine into DNA while exhibiting normal growth. The deficiency in thymidine incorporation in *E. coli tdk* can be complemented by heterologous sources of *tdk* (12, 25). Because tdk is essential for the utilization of thymidine, we considered the possibility that gp1.7 might have thymidine kinase activity. Therefore, we examined the inhibition of T7 growth by ddT in *E. coli* HMS89 *tdk*. WT T7 was able to grow on *E. coli* HMS89 *tdk* at concentrations of ddT up to 1.5 mM, with only a minor effect on the plating efficiency; this concentration of ddT reduces the plating efficiency of WT T7 by a factor of 1,000 on *E. coli* HMS89 (Fig. 6). No incorporation of [^{3}H]thymidine into T7 DNA could be detected after infection of *E. coli* HMS89 *tdk* with T7 phage (data not shown). Thus, gp1.7 was unable to substitute for the host tdk, and phage T7 apparently does not encode a tdk.

Discussion

In the present work, we have obtained data implicating the relatively uncharacterized gene *1.7* in nucleotide metabolism. T7 phage growth is strongly inhibited by the presence of ddT in the medium. It is well established that T7 DNA polymerase incorporates ddNMPs more efficiently than other homologous DNA polymerases such as *E. coli* DNA polymerase I or *Taq* polymerase (14, 15). Incorporation of ddNMPs results in chain termination, effectively halting DNA synthesis. We originally sought to exploit this property to screen for mutations in gene *5*, the structural gene for T7 DNA polymerase, which confers discrimination against ddNTP. Surprisingly, when we isolated T7 phage that could grow in the presence of ddT, the majority harbored mutations in gene *1.7* rather than in gene *5*. By following DNA synthesis *in vivo* as measured by [^{3}H]thymidine incorporation into DNA, we confirmed that ddT kills T7 phage by inhibiting DNA synthesis.

It is interesting that our first screen did not unveil the alteration in T7 DNA polymerase that is responsible for distinguishing between dNTPs and ddNTPs (15). In the present work, we eliminated the more frequently arising mutation in gene *1.7* by having gp1.7 produced from a plasmid during the screen. In this screen we find that all of the mutant phage have a single alteration in T7 gene *5* protein, the transversion of adenine at nucleotide 1577, the second base of codon (TAT) encoding tyrosine-526, to thymine, results in a new codon (TTT) encoding phenylalanine. This single alteration in T7 DNA polymerase leads to discrimination against the incorporation of ddTTP by ≈8,000-fold, compared with dTTP (14, 15). Our data indicate that mutations in gene *1.7* arise ≈100,000-fold more frequently than does the single nucleotide change in gene *5*. A variety of alterations in gene *1.7*, including frameshift, missense, and nonsense mutations, result in resistance of T7 phage growth to ddT, giving rise to its observed high mutation frequency. Our data suggest that ddT is inhibitory to T7 growth because it is incorporated into phage DNA by T7 DNA polymerase. Thus, it seems likely that resistance of T7 phage defective in gene *1.7* to ddT reflects a defect in the conversion of ddT to ddTTP because these mutant phage encode WT T7 DNA polymerase, which readily uses ddTTP.

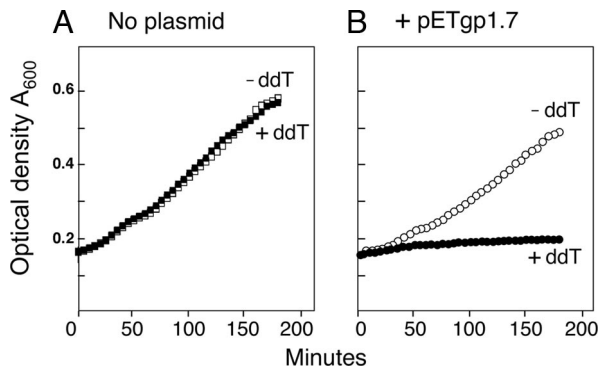

Fig. 5. Gene *1.7* confers *E. coli* sensitivity to ddT. *E. coli* HMS89(DE3) (*A*) and *E. coli* HMS89(DE3)/pETgp1.7 (*B*) were grown in a 96-well microplate containing LB medium either in the presence (■, ●) or absence (□, ○) of 1 mM ddT. Cells were induced with 1 mM IPTG at 0 min, and then the optical density was monitored by using a M5 plate reader (Molecular Devices).

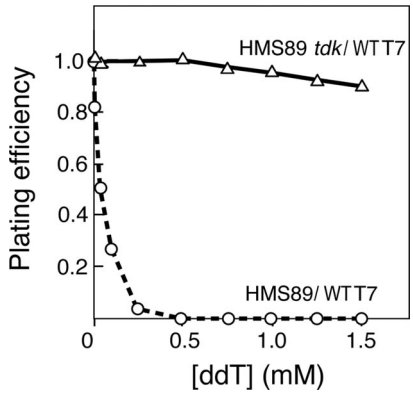

Fig. 6. *E. coli* thymidine kinase is required for inhibition of T7 phage by ddT. WT T7 phage was used to infect either HMS89 (○) or HMS89 *tdk* (△) in the presence of 0–1.5 mM ddT. Plating efficiencies were calculated as described in the legend of Fig. 1.

We find that gp1.7 produced from a plasmid also renders uninfected *E. coli* sensitive to ddT. DNA synthesis in uninfected cells harboring the plasmid pETgp1.7 is inhibited by ddT, as measured by [^{3}H]thymidine incorporated into DNA, mirroring the observations in T7-infected cells. Clearly, other phage-encoded proteins or the process of infection is not critical for the function of gene *1.7*.

Little is known about the function of gene *1.7*. It is a class II gene, synthesized between 6 and 15 min after infection (1). In the present work, we have constructed T7 phage with a deletion of entire gene *1.7* by replacing gene *1.7* with the *E. coli trxA* gene. The resulting phage appears to grow normally but is resistant to high concentrations of ddT. The fact that gene *1.7* is not required for growth by phage T7 agrees with previous studies (1). However, our studies on *in vivo* DNA synthesis in T7-infected cells do reveal a slight delay in the onset of maximal DNA synthesis in the absence of gp1.7. This finding is in agreement with a report that inactivation of gene *1.7* had a quantitative effect on T7 growth, in that the eclipse period and time required for maximum progeny were longer than those observed for WT T7 (26).

Although the data we present here implicate gene *1.7* in nucleotide metabolism, the exact function of the protein it encodes remains a mystery. For phage growth to be inhibited by ddT, three major events must occur. First, ddT must enter into the cell. Second, it must be converted to dTTP. Finally, T7 DNA polymerase must incorporate ddTTP into DNA.

One possibility is that gp1.7 is a tdk, catalyzing the conversion of dT or ddT into the corresponding nucleoside monophosphate. However, our data do not support this role. *E. coli* tdk, an essential enzyme of the thymine salvage pathway (23), is encoded by the *tdk* gene. WT T7 phage is no longer sensitive to the presence of ddT in the absence of host *tdk*. Moreover, the incorporation of [^{3}H]thymidine into DNA could not be detected in *E. coli* HMS89(DE3) *tdk* containing the pETgp1.7 plasmid. This finding agrees with previous data that although a phage-specific tdk is found in T-even phage, none was observed in phage T7 (12, 13). The possibility remains that gp1.7 serves as an accessory protein to facilitate or alter the specificity of a host protein such as tdk. In this case, tdk would still be required for the conversion of ddT to ddTMP, and gp1.7 would only enhance its activity or alter its substrate specificity.

DNA synthesis in T7 phage-infected cells occurs at a rapid rate. A rate-limiting step in the phage life cycle can be the accumulation of a sufficient pool of nucleoside triphosphates to be incorporated into newly synthesized DNA. Phage T7 has partially solved this problem by using the dNMPs derived from host DNA, thus bypassing the complicated step of reduction of ribonucleotides (2, 3). If any step were to be rate-limiting in T7-infected cells, then one might choose the conversion of nucleoside monophosphates to nucleoside diphosphates. If gp1.7 plays a role in this step, it is likely to be specific for dTMP because only ddT was inhibitory in the *in vivo* screen.

Sequence alignment provides no hints to the function of gene *1.7* because all of the genes with which it shares high sequence homology are also of unknown function, including the gene *1.7* from the phage Ye03-12, T3, K1F, and SP6. It is interested to note that the region of homology lies in the C terminus of the predicted proteins, the region that our deletion mapping studies show to be involved in ddT sensitivity.

Finally, other roles of gp1.7 could explain the observed phenotype. The incorporation of ddTMP into DNA depends not only on the ability of the polymerase to incorporate these analogs but also on the cellular concentration of ddTTP relative to dTTP. A reduction of the dTTP pool by gp1.7, for example, would increase the frequency of incorporation of ddTMP into DNA, a scenario that does not require any effect of gp1.7 on ddT metabolites. Alternatively, inhibition of an enzyme that makes *E. coli* efficiently use exogenous thymidine could also increase the ability of using ddT. Our data, however, do not support the role of gp1.7 as an inhibitor of a host metabolic enzyme because T7Δ1.7 is insensitive to ddT in *E. coli* lacking the *deoA*, *deoB*, *deoC*, *deoD*, *yjjG*, *dcd*, or *thyA* genes (data not shown). Mutations in any of these genes are believed to increase the ability of *E. coli* to use exogenous thymidine (23, 27–29). Elucidation of the exact function of gp1.7 must await its biochemical characterization.

Materials and Methods

Bacterial and Phage Strains. *E. coli* HMS89 (*xth-1 thi argE mtl xyl str-R ara his galK lacY proA leu thr tsx supE*) was used for all dideoxynucleoside assays. *E. coli* DH5α (Invitrogen) was used for cloning T7 gene *1.7*. *E. coli* A307 (*trxA*) was used for construction of T7Δ1.7-2. T7 phage LG37, LG30, D24, and LG26 were used to map the region in T7 responsible for sensitivity to ddT (20).

Determining Sensitivity of T7 Phage to Dideoxynucleosides. WT T7 phage were used to infect a culture of *E. coli* HMS89 in the presence of varying concentrations of dideoxyadenosine, dideoxycytidine, or dideoxythymidine. Dideoxynucleosides (ICN) were added to either Davis minimal (Difco) or LB medium soft agar (0.7%) at 45°C before adding *E. coli* cells and phage. The mixture was overlaid onto either LB or Davis minimal medium agar plates. Plates were incubated at 37°C for 3 h. Plating efficiencies were determined by dividing the number of plaques observed in the presence of a dideoxynucleoside by the number observed in its absence.

Isolating T7 Phage Resistant to ddT and Sequencing Their DNA. Plaques were isolated from on lawns of *E. coli* growing in the presence of 2.5 mM ddT. The phage from a number of these plaques were picked and placed into a solution containing 0.9% NaCl solution and left overnight at 4°C. Two-milliliter cultures of *E. coli* HMS89 were grown overnight in minimal medium. The medium was then supplemented with 2.5 mM ddT, and the cells were infected with the isolated phage at 37°C. Supernatants of the culture were collected by centrifugation at 12,000 $\times$ *g* for 1 h at 4°C. Phage were precipitated by the addition of 0.25 volume of 15% PEG 8000 and 2.5 mM NaCl for 20 min at 0°C followed by centrifugation at 12,000 $\times$ *g* for 1 h. Phage were resuspended in 1 M NaCl and stored at 4°C. Phage DNA was prepared by extraction twice with phenol, and DNA was then precipitated by ethanol. The region of gene *1.7* from each mutant was amplified by PCR and sequenced.

Construction of pETgp1.7 Expression Vector. Gene *1.7* was amplified from T7 phage DNA by using forward and reverse primers containing NdeI and BamHI restriction sites, respectively, at their 5′ ends. The resulting 606-bp fragment was inserted into the expression vector pET17b (Novagen) between the NdeI and BamHI sites. The resulting plasmid, pETgp1.7, was transformed into *E. coli* strain DH5α. The pETgp1.7 plasmid was sequenced to ensure that no mutations were introduced during the cloning procedures.

Construction of T7Δ1.7-2 Phage. To construct T7Δ1.7-2, gene *1.7* was substituted by the *trxA* gene of *E. coli* at the same position of the T7 genome. A DNA fragment containing the coding sequence of gene *trxA* of *E. coli* and flanking regions of 234 and 116 bp of T7 gene *1.6* and *1.8*, respectively, was generated by using PCR. The DNA fragment was inserted into the plasmid pT7-7 between the EcoRI and BamHI sites. In the resulting plasmid, pT7-TrxA, the expression of the *trxA* gene is under the control of a T7 RNA promoter. This plasmid was transformed into *E. coli* A307 *trxA*, which lacks the *trxA* gene. A307 *trxA* carrying pT7-TrxA was infected with WT T7 phage. Surviving phage were used to infect *E. coli* A307 *trxA* not carrying pT7-TrxA plasmid. Because trxA is essential for T7 phage growth, only phage that contained the *trxA* gene in the place of gene *1.7* were able to grow. T7 phage from individual plaques were purified, and the deletion of gene *1.7* was confirmed by DNA sequencing.

Construction of *E. coli HMS89 tdk* and Other Mutated Genes. *E. coli* HMS89 lacking the *tdk* gene was constructed following the protocol described in the Quick and Easy *E. coli* gene deletion kit (Gene Bridges). *E. coli* HMS89 carrying the Red helper plasmid pKD46 was grown in 5 ml of LB culture at 30°C until the A_{600} = 0.4. The culture was induced at 37°C by adding L-arabinose to a final concentration of 0.4% for 1 h to express the λRed/ET recombinases. Cells were harvested and made electrocompetent. A DNA fragment containing the kanamycin resistance gene flanked by 250 bp upstream and 250 bp downstream of the *tdk* gene was generated by PCR. Four hundred nanograms of purified DNA fragment was electroporated into the electrocompetent cells by using the *E. coli* Pulser (Bio-Rad). The cells were then suspended in 1 ml of LB

medium, incubated at 37°C for 2 h, then spread on LB agar plates containing 50 μg/ml kanamycin. *E. coli* cells with deletions of the *deoA*, *deoB*, *deoC*, *deoD*, *yjjG*, *dcd*, or *thyA* genes were constructed by using the same procedure.

Complementation Assay. The plasmid pETgp1.7 was transformed into *E. coli* HMS89. In this plasmid, the expression of gene *1.7* is under the control of the T7 RNA promoter, so gene *1.7* will be expressed when the cells are infected by T7 phage. An overnight culture of *E. coli* HMS89/pETgp1.7 was grown in LB medium supplemented with varying concentrations (0–2.5 mM) of ddT and then infected with either WT or T7Δ1.7-2 phage. The infected *E. coli* was plated onto LB agar plates in soft agar and grown at 37°C for 3 h.

Inhibition of *E. coli* Growth by ddT. *E. coli* HMS89 was lysogenized with the λ phage DE3 by using the λDE3 lysogenization kit (Novagen). This strain expresses T7 RNA polymerase upon induction by IPTG. Hosts HMS89(DE3) and HMS89(DE3)/pETgp1.7 were grown in LB medium to a density of 3×10^8 cells per ml. Cells (180 μl) were aliquoted into individual wells of a 96-well microplate containing increasing concentrations (0–7.5 mM) of ddT. IPTG was added to final concentration of 1 mM. The microplate was then placed into a M5 plate reader (Molecular Devices) to monitor the A_{600} at 37°C. The percentage of inhibition of cell growth was determined by dividing the difference in A_{600} observed in the presence of ddT from that observed in its absence, by the A_{600} observed for cells grown in the absence of ddT.

***In Vivo* DNA Synthesis.** T7 DNA synthesis was measured by the incorporation of [^{3}H]thymidine into viral DNA (30). *E. coli* HMS89 was infected by either WT or T7 ddT^{R}1 phage at a multiplicity of infection of 7. When present, ddT was added to a final concentration of 0.45 mM. The incorporation of [^{3}H]thymidine into DNA was measured by binding to DE81 discs followed by liquid scintillation counting as described in ref. 30.

Screening for ddT Resistance Mutations in T7 Gene *5*. *E. coli* HMS89 carrying plasmid pETgp1.7 was used for infection of WT T7 in the presence of 1 mM ddT. Ninety-six T7 phage resistant to ddT were suspended in the individual wells of a microplate containing LB medium. These mutants were then stamped on plates containing 1 mM ddT and *E. coli* HMS89 that contains the plasmid pGP5-3, which produces the T7 DNA polymerase under the control of a T7 promoter (30). Phage that were not resistant to ddT in the presence of pETgp1.7 but were inhibited by ddT in the presence of pGP5-3 were picked up for further analysis. The gene *5* region from nine of these mutants was sequenced.

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