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Analysis of T4 Bacteriophage Deletion Mutants That Lack *td* and *frd* Genes

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The roles of bacteriophage T4-encoded thymidylate synthase and dihydrofolate reductase as virion structural components have been further investigated. Two mutants, *del*(63-32)7 and *del*(63-32)9, bearing deletions in the gene 63 to 32 region of the T4 genome, were characterized by Southern blotting analysis, as well as by enzyme and immunological assays. Our results have confirmed the original report of Homyk and Weil (Virology 61:505–523, 1974) that *del*7 and *del*9 each carries a deletion of about 4.0 kilobases, which totally eliminates the *frd* gene, encoding dihydrofolate reductase, and the *td* gene, encoding thymidylate synthase. With the well-characterized deletion mutants, along with newly prepared antisera against T4-encoded thymidylate synthase and dihydrofolate reductase, we have reevaluated the experimental results supporting the idea that T4-induced dihydrofolate reductase and thymidylate synthase are essential T4 baseplate components and antigenic determinants of phage particles. These deletion mutant phages are not targets for neutralization by antisera against either dihydrofolate reductase or thymidylate synthase purified from cloned genes. Furthermore, these newly prepared antisera also cannot neutralize the infectivity of T4D. Those results suggest that the phage-neutralizing components in the old antisera used in the earlier studies were not antibodies against either dihydrofolate reductase or thymidylate synthase but were antibodies against minor components of the purified enzyme preparations. Study of the biological properties of the deletion mutants indicates that T4-induced thymidylate synthase and dihydrofolate reductase play significant roles in growth of the phage beyond their known roles in nucleotide biosynthesis, even though they are apparently not essential for phage viability. The deletion mutants should be useful in defining these roles.

The genome of bacteriophage T4 encodes most of its own enzymes for deoxyribonucleotide biosynthesis and DNA replication. Some of the enzymes duplicate and augment the functions of host cell enzymes, helping thereby to support the high rate of DNA synthesis in T4 phage-infected cells. Thymidylate synthase and dihydrofolate reductase, which are involved in thymine nucleotide biosynthesis, are two such enzymes. In addition to their catalytic functions, the two enzymes are also present in the phage tail baseplate (3, 10, 16). That these proteins play structural roles is supported mainly by four lines of evidence: (i) detection of low levels of enzyme activities in purified viral particles (8–10, 19); (ii) neutralization of T4 phage infectivity by antiserum against either of the enzymes (3, 17); (iii) the observation that genes coding for the two enzymes are determinants of physical properties of the virion, including heat lability (3, 7–9, 16); and (iv) the presence in baseplates of a folate compound, dihydropteroylhexaglutamate, and evidence that it is bound to these enzymes in situ (21, 25).

Some years ago, we studied three deletion mutants that had been characterized by Homyk and Weil (5)—*del*(63-32)1, *del*(63-32)7, and *del*(63-32)9—hereafter called *del*1, *del*7, and *del*9, respectively. According to DNA heteroduplex mapping, two of the mutants, *del*7 and *del*9, are deleted for both the *td* gene, encoding thymidylate synthase, and the *frd* gene, encoding dihydrofolate reductase. The deletion mutants were used in attempts to confirm that thymidylate synthase and dihydrofolate reductase are T4 baseplate components. When neutralization of infectivity was studied, unexpectedly, the mutants were found to be even more readily neutralized than wild-type T4D by antisera against either T4 thymidylate synthase or dihydrofolate reductase (3, 19). Moreover, deletion mutant-infected *Escherichia coli* cell extracts contain proteins which can cross-react with the antisera, as shown by immunodiffusion (3, 19). These results are unexpected if the proteins are completely missing from the virion. One possible explanation for these paradoxical results was that the *td* gene and *frd* gene are not completely deleted in the mutants, and the remaining parts of these genes can still produce cross-reacting materials to interact with thymidylate synthase and dihydrofolate reductase antibodies (20).

To understand the apparent paradox and to elucidate the structural roles of thymidylate synthase and dihydrofolate reductase, it is important to carefully define the deletion mutants. In this investigation, we characterized the mutants with Southern blotting analysis, along with immunological and enzyme assays. We also made new antisera to T4 thymidylate synthase and dihydrofolate reductase purified from *E. coli* cells containing either the cloned T4 *td* gene or the *frd* gene. With the well-characterized T4 deletion mutants and newly made antibodies, we reexamined the experiments supporting the idea that thymidylate synthase and dihydrofolate reductase are essential T4 baseplate components and antigenic determinants of phage particles.

(This research was conducted by Y. Wang in partial fulfillment of the requirements for the Ph.D. degree from the Oregon State University, Corvallis.)

MATERIALS AND METHODS

Cell and bacteriophage strains. *E. coli* B (wild-type) and wild-type phage strain T4D have been maintained in this laboratory for some time. Mutant strains bearing deletions

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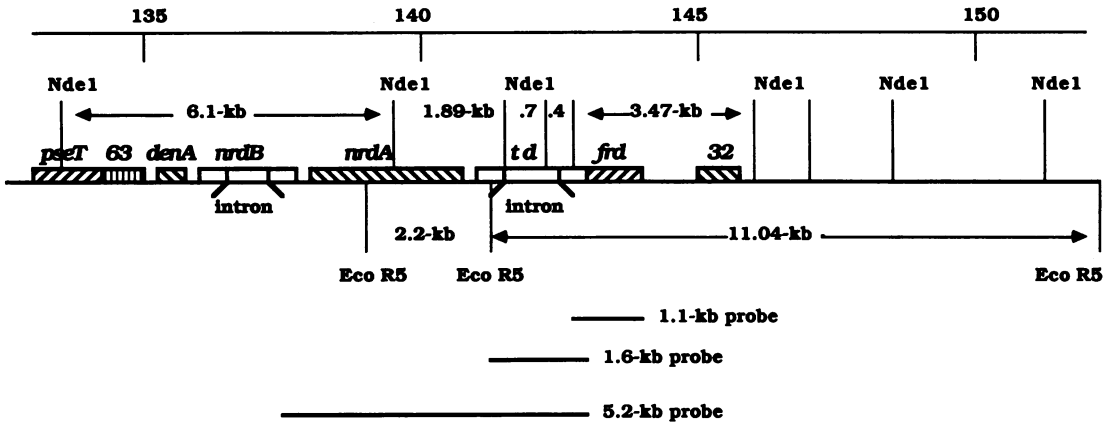

FIG. 1. Restriction map of the gene 63 and 32 region of bacteriophage T4 DNA, showing also the restriction fragments used as hybridization probes in this study. The upper index shows distance in kilobases from a reference point on the T4 genomic map, namely, the *r*IIA/*r*IIB cistron divide.

between genes 63 and 32 were obtained from T. Homyk, Jr. (5), Vanderbilt University; these included *del7* and *del9*, the objects of this study. The *frd* gene recombinant plasmid pSP19 was constructed in this laboratory (22). *E. coli* MB151, a strain containing the *td* gene recombinant plasmid pKTd, was obtained from Marlene Belfort, New York State Department of Health (1).

Purification of T4 phage DNA. The phage particles in a crude lysate were concentrated 10-fold by centrifugation at 23,000 × *g* for 2 h, and were then suspended in 0.1 volume of M9 salts solution. The suspension was centrifuged at 4,000 × *g* for 10 min to remove the remaining cellular debris. Phage particles from the supernatant were collected by centrifugation at 35,000 × *g* for 30 min and suspended in 0.05 volume of M9 salts solution. DNA was isolated from the purified phage suspension by phenol extraction, followed by ethanol precipitation (12).

Southern blotting analysis. T4 DNA was digested with restriction enzymes and fractionated in a 0.7% agarose gel as described previously (12). The DNA was transferred to a nylon membrane (Bio-Rad) and hybridized to a DNA probe that had been ^{32}P labeled by the hexamer primer method (4).

Western blotting analysis. Approximately 50 μg of protein of each sample was electrophoretically fractionated in a 10% sodium dodecyl sulfate-polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Dilutions (1:1,000) of primary and secondary antibodies were used in immune reactions as described previously (2).

Antiserum inactivation of phage infectivity. Antiserum inactivation of phage infectivity was done as described by Capco and Mathews (3), except that the experiment with dihydrofolate reductase antiserum was carried out with antiserum diluted 10-fold instead of 50-fold.

Dihydrofolate reductase inactivation by its antiserum. A 50-μl portion of T4-infected *E. coli* cell extract was incubated with 20 μl of anti-DHFR in a 1.0-ml dihydrofolate reductase enzyme assay reaction mixture, complete except for NADPH, for a period of time at room temperature. The enzyme activity remaining after antiserum treatment was assayed as described by Mathews and Sutherland (18).

RESULTS

Southern blotting analysis of deletion mutants with T4 dihydrofolate reductase gene probe. The deletions in the 63 to 32 region of the T4 genome were originally mapped by DNA heteroduplex mapping (5). The *td* gene, encoding thymidylate synthase, and the *frd* gene, encoding dihydrofolate reductase, are located in this region. The results indicated that both the *frd* gene and the *td* gene were deleted completely, or nearly so, in both *del7* and *del9*. We used Southern blotting and took advantage of the availability of cloned *frd* gene and *td* genes as probes to map the deletions more precisely.

For Southern blotting analysis, T4 genomic DNA must be digested and the fragments resolved on agarose gels. However, T4 DNA contains glucosylated hydroxymethyldeoxycytidylate residues. This modification makes it resistant to digestion by most commonly used restriction enzymes. So far only six restriction enzymes are known to cleave modified T4 DNA (11; E. Kutter and B. Guttmann, T4 News **1**:3–19, 1986), but some of them cannot digest it to completion. For Southern blotting analysis of the deletion mutants, we selected the restriction enzymes *Eco*RV and *Nde*I, based on their ability to digest unmodified DNA to near completion and to produce fragments of distinguishable sizes.

The *Eco*RV digestion pattern is particularly useful because there is a distinctive pattern of cleavage sites in the region between genes 63 and 32. This is shown in Fig. 1, which also shows the cloned fragments we used for our Southern blotting analysis. Three *Eco*RV fragments cover the whole 63 to 32 region, and an 11.04-kilobase (kb) fragment contains the whole *frd* gene. Upon digestion of wild-type and deletion mutant DNAs with *Eco*RV, followed by electrophoresis, we observed DNA differences between wild-type and deletion mutant DNAs, as shown in Fig. 2. The 11.04-kb fragment is absent from the deletion mutant patterns. Since *del7* and *del9* also contain a second deletion in the *r*II gene (5), a 7.5-kb *Eco*RV fragment that contains the *r*II cistrons was also absent from the deletion mutant DNAs.

The T4 *frd* gene has been cloned and expressed in our laboratory (22, 23). A 1.1-kb *Hin*dIII fragment containing the *frd* gene was excised from the recombinant plasmid pSP19 and used as the probe for Southern blotting analysis (Fig. 3a). When *Eco*RV-digested T4 DNA was hybridized with the radioactive-labeled *frd* gene probe, the probe hybridized to the wild-type 11.04-kb DNA fragment but not to any fragment in *del7* or *del9* DNA. The probe also hybridized a higher-molecular-weight band, probably resulting from incomplete digestion of T4D DNA. In any case, this experi-

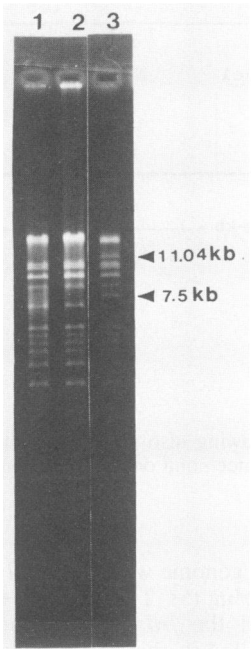

FIG. 2. *Eco*RV-digested T4 DNAs fractionated on a 0.7% agarose gel. Lane 1, *del*7 DNA; lane 2, *del*9 DNA; and lane 3, wild-type T4D DNA, stained with ethidium bromide.

ment shows that the *frd* gene is totally deleted in mutants *del*7 and *del*9.

In order to more precisely map the deletions, we used another restriction enzyme, *Nde*I. One problem with this enzyme is that it digests T4 DNA into so many fragments that the difference between wild-type and mutant T4 DNAs cannot be observed on an ethidium bromide-stained agarose gel. Wild-type and mutant T4 DNAs were digested with *Nde*I and hybridized with the same radiolabeled *frd* gene probe after transfer to a nylon membrane. According to the *Nde*I restriction map in Fig. 1, the *frd* gene lies in a 3.47-kb DNA fragment in wild-type T4 DNA. As expected, Fig. 3b shows that the *frd* gene probe hybridized to a fragment of the correct size in wild-type T4 DNA. Again, nothing was hybridized by the *frd* gene probe in the deletion mutant DNAs.

Southern blotting analysis of deletion mutants with T4 thymidylate synthase gene probe. To see if the *td* gene is also deleted in the mutants *del*7 and *del*9, we used a *td* gene probe to hybridize the same membrane onto which *Nde*I-digested DNA was transferred. The 1.6-kb *td* gene probe was from a recombinant plasmid pKTd (1), and it contains the whole *td* gene and a small portion of the *frd* gene. As shown in Fig. 1, the 1.6-kb DNA fragment contains four *Nde*I restriction fragments in wild-type T4 DNA. The autoradiogram in Fig. 3c shows what we expected for wild-type T4 DNA: four DNA fragments of the right size were hybridized by the *td* gene probe. It also shows that nothing in *del*7 or *del*9 DNA was hybridized by the *td* gene probe. It clearly shows that not only the *frd* gene but also the *td* gene is completely deleted in these mutants.

Southern blotting analysis with a 5.2-kb T4 DNA fragment to precisely map the ends of the deletions. A 5.2-kb *Hin*dIII DNA fragment used to map the deletion ends extends from the middle of the *frd* gene to the middle of the *nrdB* gene, which codes for the small subunit of T4 ribonucleotide reductase. There are five *Nde*I restriction fragments in this 5.2-kb fragment, according to the restriction map of wild-type phage DNA shown in Fig. 1. When the same *Nde*I-digested T4 DNA immobilized on the membrane was hybridized with the 5.2-kb DNA probe, five bands were seen in wild-type T4 DNA, as expected (Fig. 4). In the deletion mutants, a 6.1-kb fragment was hybridized similarly to the one seen in wild-type DNA. In addition a new 2.5-kb fragment was hybridized in the deletion mutants. The new

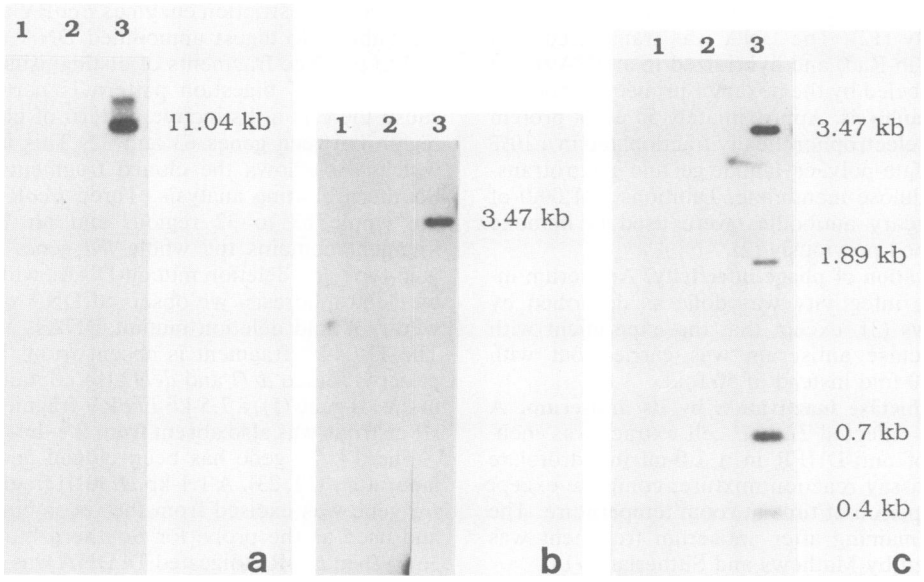

FIG. 3. Southern blotting analysis of restriction fragments of T4 DNAs. (a) *Eco*RV-digested DNAs analyzed with the 1.1-kb *frd* gene probe (shown in Fig. 1). Fragments were separated on an agarose gel and transferred to a nylon membrane. (b) Analysis of *Nde*I-digested T4 DNAs with the *frd* gene probe, carried out identically as described in panel a. (c) Analysis of *Nde*I-digested T4 DNAs with a 1.6-kb *td* gene probe. *Nde*I-digested T4 DNAs immobilized on a nylon membrane were hybridized with the 1.6-kb *Eco*RV-*Hin*dIII DNA fragment, shown in Fig. 1. For panels a, b, and c: lane 1, *del*7 DNA; lane 2, *del*9 DNA; and lane 3, T4D DNA.

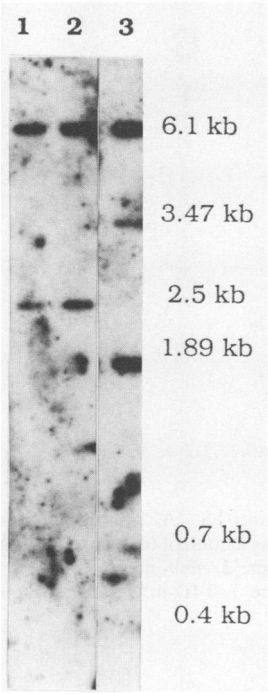

FIG. 4. Mapping of the deletion ends with a 5.2-kb *Hin*dIII T4 DNA fragment (shown in Fig. 1). *Nde*I-digested T4 DNAs immobilized on a nylon membrane were hybridized with the radiolabeled 5.2-kb fragment and exposed to autoradiography. Lane 1, *del*7 DNA; lane 2, *del*9 DNA; and lane 3, T4D DNA.

fragment is generated by joining two partially deleted *Nde*I fragment remainders in the mutant DNAs. The difference between 2.5 kb and the sum of the sizes of the four missing fragments (0.4 + 0.7 + 1.89 + 3.47) is 4.0 ± 0.1 kb, so this represents the length of the deletion. Since one end of the deletion lies to the right of 143.94 on the T4 genomic map (11), while the other end must extend into gene *nrdA*, to account for the ribonucleotide reductase-defective phenotype (5), the imprecision in locating the end points is as indicated in Fig. 5 (about 0.6 kb).

Characterization of the deletion mutants at the protein level. We carried out Western blotting analysis with antisera to T4 thymidylate synthase and dihydrofolate reductase purified from *E. coli* strains carrying cloned genes. Neither anti-TS nor anti-DHFR reacted with corresponding proteins in the deletion mutants, as shown in Fig. 6. Enzymatic assays were carried out with deletion mutant-infected cell extracts. Figure 7 confirms our earlier observation that no T4-induced thymidylate synthase or dihydrofolate reductase activities can be detected in the mutant-infected cell extracts.

Phage infectivity neutralization experiments with deletion mutants and antisera against cloned T4 gene products. Our previous studies showed that the infectivity of phages bearing deletions in the 63 to 32 region can be neutralized by antisera against T4 thymidylate synthase or dihydrofolate reductase and that mutant-infected cell extracts contain proteins that cross-react with both antisera (3, 19). These results were difficult to reconcile with the apparent absence of *td* and *frd* genes in these strains. One possible explanation was that the *frd* gene and *td* gene were not totally deleted, and truncated proteins could still be produced and incorporated into the baseplate in the deletion mutants (3, 19). Our data here show that this is not possible; both the *td* gene and the *frd* gene are completely absent from the mutant DNAs. The current results made us suspect the antisera used in the early studies. Even though the antisera were generated from electrophoretically homogeneous proteins purified from T4-infected cells and even though immunodiffusion experiments suggested the presence of antibodies to single proteins, we could not eliminate the possibility that trace amounts of other T4 protein contaminants also had raised antibodies in the sera. In other words, the antibody which neutralizes phage infectivity might not react against dihydrofolate reductase or thymidylate synthase but against some other T4 proteins in the old antisera.

Accordingly, we made new antisera against T4 dihydrofolate reductase and thymidylate synthase. The proteins used to make new antibodies were from cloned T4 gene products, not from T4-infected *E. coli* cells. Even though the proteins purified from cells containing cloned T4 genes may be contaminated with trace amount of *E. coli* proteins, the preparations cannot contain any other T4 proteins.

With the newly generated antibodies, we carried out phage infectivity neutralization experiments of the kind carried out a decade ago in our laboratory. If thymidylate synthase and dihydrofolate reductase are the baseplate components that serve as antigenic determinants of phage particles, antibodies against the cloned gene products should be able to neutralize infectivity of wild-type T4 phage but not that of the deletion mutants. Figure 8 shows the result of the experiment. Unexpectedly, dihydrofolate reductase antiserum not only failed to neutralize infectivity of deletion mutants, but also that of T4D. We observed the same result with thymidylate synthase antiserum (data not shown). To optimize experimental conditions for phage infectivity neu-

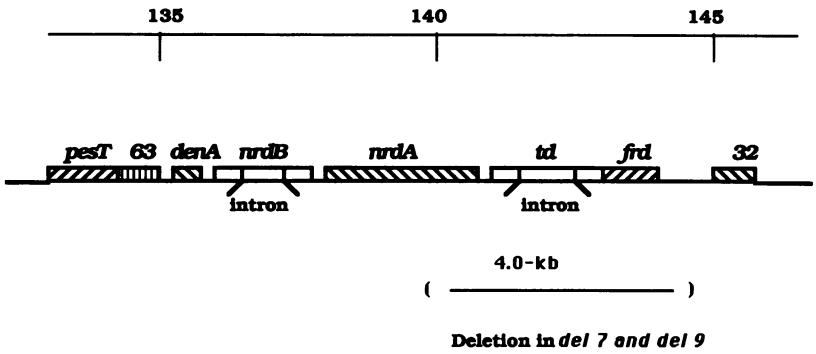

FIG. 5. Deletion in 63-32 region of *del*7 and *del*9 DNA, mapped from the data of Fig. 3 and 4.

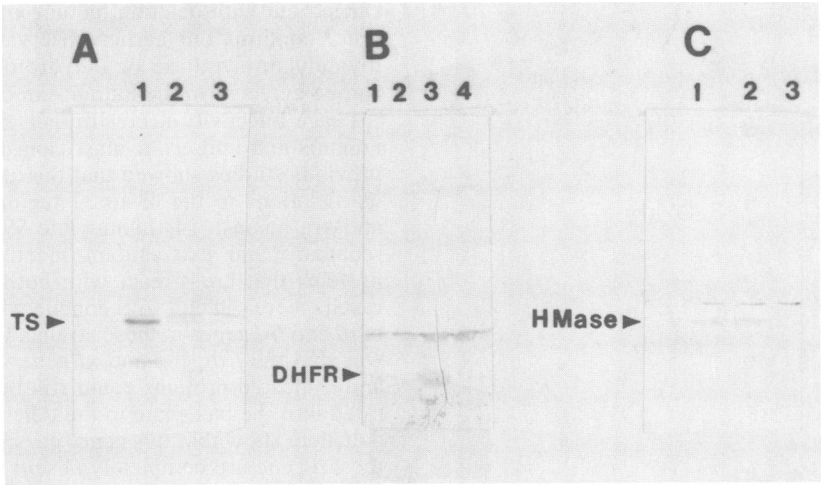

FIG. 6. Western blotting with T4 phage-infected *E. coli* cell extracts, anti-DHFR, and anti-TS. (A) Immune reaction with anti-TS. (B) Immune reaction with anti-DHFR. (C) Immune reaction with anti-dCMP hydroxymethylase as a control to ensure that infection had occurred in all cells. The cell extract applied to each lane of panels A and C: lane 1, T4D-infected; lane 2, *del*9-infected; lane 3, uninfected cell. The cell extract applied to each lane of panel B: lane 1, *del*7-infected; lane 2, *del*9-infected; lane 3, T4D-infected; and lane 4, uninfected. The proteins were identified by their molecular weights, shown by arrows.

tralization, we have tried different phage dilutions as well as different antiserum dilutions. We did not see any neutralization activity in those conditions.

To further test our experimental conditions, our 17-year-old dihydrofolate reductase antiserum was used as a control. Figure 9 shows that the old antiserum has retained some ability to neutralize phage infectivity and that *del*7 is more sensitive than T4D, but under the same experimental conditions the new antiserum contains no phage-neutralizing activity. Although unlikely, we must consider the possibility that the old antiserum contains a higher concentration of DHFR-specific antibodies than does the new antiserum, so that we can only observe the neutralization activity of old antiserum. To evaluate this idea, we tested the ability of each antiserum to inhibit enzyme activity. However, Fig. 10 shows that within the limits of our experimental conditions, the two antisera are equal in their ability to inactivate dihydrofolate reductase enzyme activity.

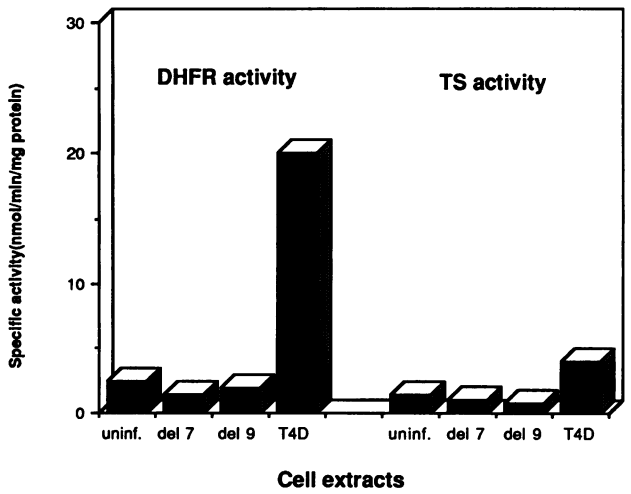

FIG. 7. Dihydrofolate reductase and thymidylate synthase enzyme assays with wild-type and deletion mutant-infected *E. coli* cell extracts. A 50-μl portion of each cell extract was used as the enzyme source, and the assays were run as described previously (18).

Biological properties of *del*7. As noted earlier, the *frd* and *td* gene products were shown to be present in the T4 phage baseplate in work both by Kozloff and colleagues and from our laboratory (3, 7, 8–10, 16, 17, 19). The viability of deletion mutants that completely lack these genes and their products calls into question whether the two proteins play essential roles as baseplate components. To be sure, phages containing these deletions grow quite poorly (5). We have not carried out extensive biochemical analyses of the defective phenotypes associated with these deletions for two reasons: (i) the phages grow so poorly that it is difficult to prepare lysates of sufficiently high titer for biochemical experiments; and (ii) because dihydrofolate reductase and thymidylate synthase both participate in DNA synthesis (13–15), it may be difficult deciding whether a defective phenotype results from defective DNA metabolism or baseplate function. Nevertheless, we have carried out a few preliminary biological experiments. Figure 11 shows that *del*7 adsorbs to its host at the same rate as T4D, although measurements of DNA injection might be a more sensitive indicator of baseplate function. Also, we have determined phage burst sizes, shown in Table 1. *del*7 is severely restricted in its growth on *E. coli* B. Since *del*7 contains the *r*II deletion, r1589, comparison with T4r1589 is more appropriate than comparison with T4D. While *del*7 does grow very poorly on *E. coli* B, the restriction of growth is much less severe in infection of a host strain carrying plasmid pPS2, which overproduces *E. coli* ribonucleotide reductase by at least 10-fold (24). This host was used in an attempt to overcome the defect in deoxyribonucleotide metabolism imposed by the *nrdA*, *frd* and *td* deletions. Since wild-type phage growth is somewhat restricted in this host, we interpret the data to mean that the poor growth of *del*7 is partly, but not completely, bypassed when the block to deoxyribonucleotide synthesis is circumvented. The results suggest that *del*7 (and *del*9) are impaired in their growth by a factor

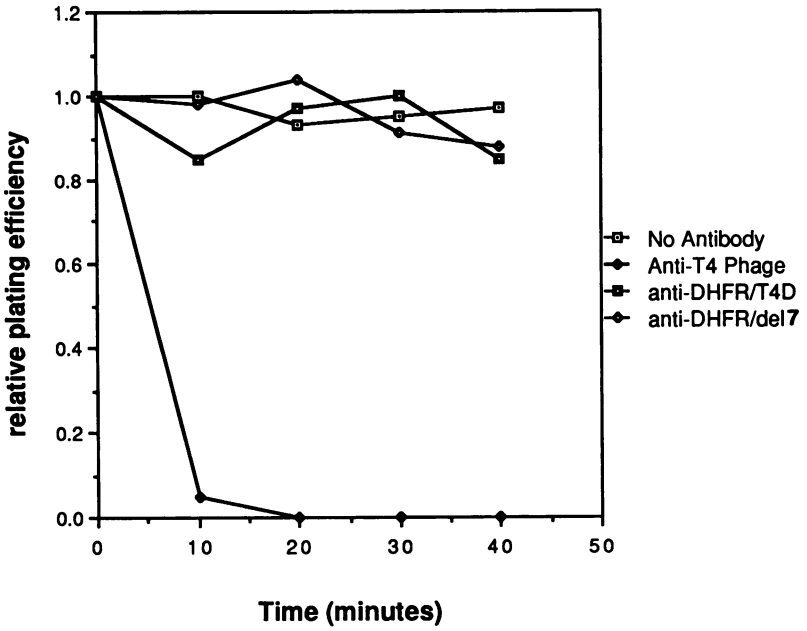

FIG. 8. Neutralization of T4 phage infectivity by antiserum against dihydrofolate reductase. Phages (10^5) were incubated with 1:10 dilution of antiserum for the indicated time intervals at 37°C. The surviving phages were diluted 1:1,000 and plated. Each datum point represents an average value obtained from two separate experiments.

over and above the limitation in deoxyribonucleotide synthesis. This factor may be the structural role played by T4 dihydrofolate reductase and thymidylate synthase, although we cannot rule out two other possibilities: (i) that the pPS2 plasmid does not completely bypass the deoxyribonucleotide deficiency or (ii) that lack of expression of another open reading frame in the 63 to 32 region is responsible for the partial restriction of growth of *del7*.

DISCUSSION

Our early attempts to define the roles of T4 thymidylate synthase and dihydrofolate reductase as virion components led to a paradoxical result: phages reported to be deleted for these genes produced proteins that cross-reacted with antisera against electrophoretically homogeneous preparations of both enzymes (3, 17, 19, 20). It was timely for us to reinvestigate this apparent paradox, using information and reagents that have become available in the 10 years since we last studied this problem—a restriction map of this region of the T4 genome, cloned fragments to use as hybridization probes, and cloned T4 *td* and *frd* genes, which allow purification of the respective enzymes totally free of contamination by other phage-coded proteins. Using these resources, we have confirmed the original report of Homyk and Weil (5)

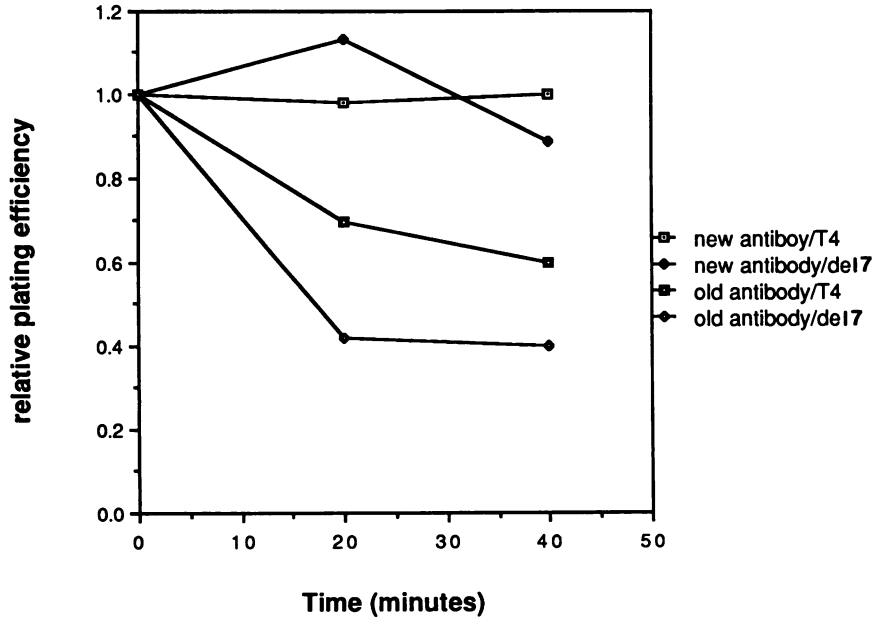

FIG. 9. Comparison of the neutralization activities of new anti-DHFR serum with old anti-DHFR serum.

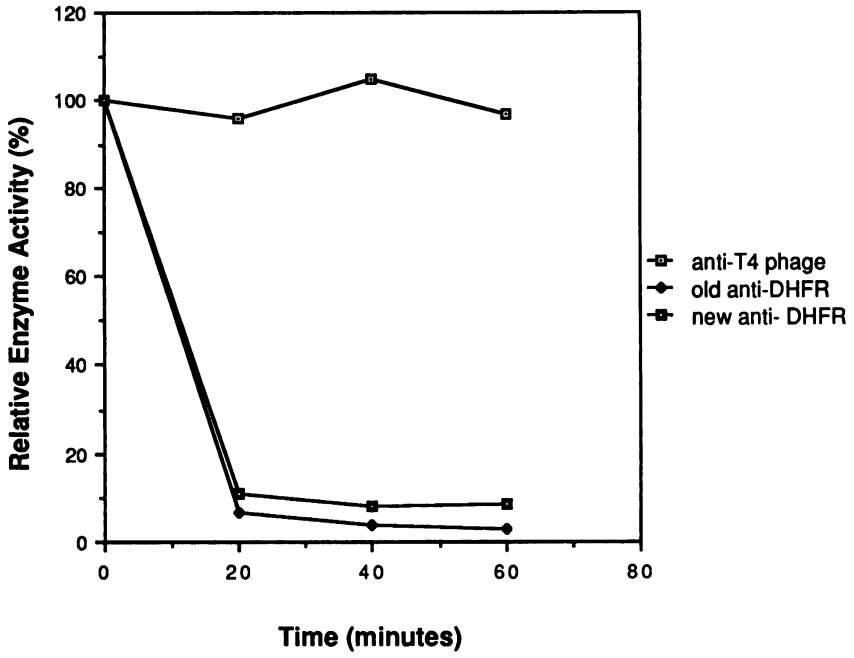

FIG. 10. Inactivation of T4 dihydrofolate reductase by its antisera. A 50-μl portion of each phage-infected *E. coli* cell extract was incubated with 20 μl of anti-DHFR in a 1-ml dihydrofolate reductase enzyme assay reaction mixture, and activity was assayed as described in Materials and Methods.

that *del7* and *del9* each carries a deletion of about 4 kb, which totally eliminates the *td* and *frd* genes and gene products. These phages are not targets for neutralization by antisera against either dihydrofolate reductase or thymidylate synthase prepared from cloned genes. Since these newly prepared antisera also fail to neutralize the infectivity of T4D, the conclusion seems inescapable that the phage-neutralizing components in our old antisera were antibodies against minor components of our purified enzyme preparations, components that could not be detected by techniques available at the time.

What, then, are the roles of thymidylate synthase and dihydrofolate reductase as virion proteins? Clearly, the enzymes do not play indispensable roles, because of the viability of deletion mutants lacking both proteins. However, the proteins are both present in wild-type phage particles, and almost certainly not as adventitious components. First, both proteins are determinants of physical

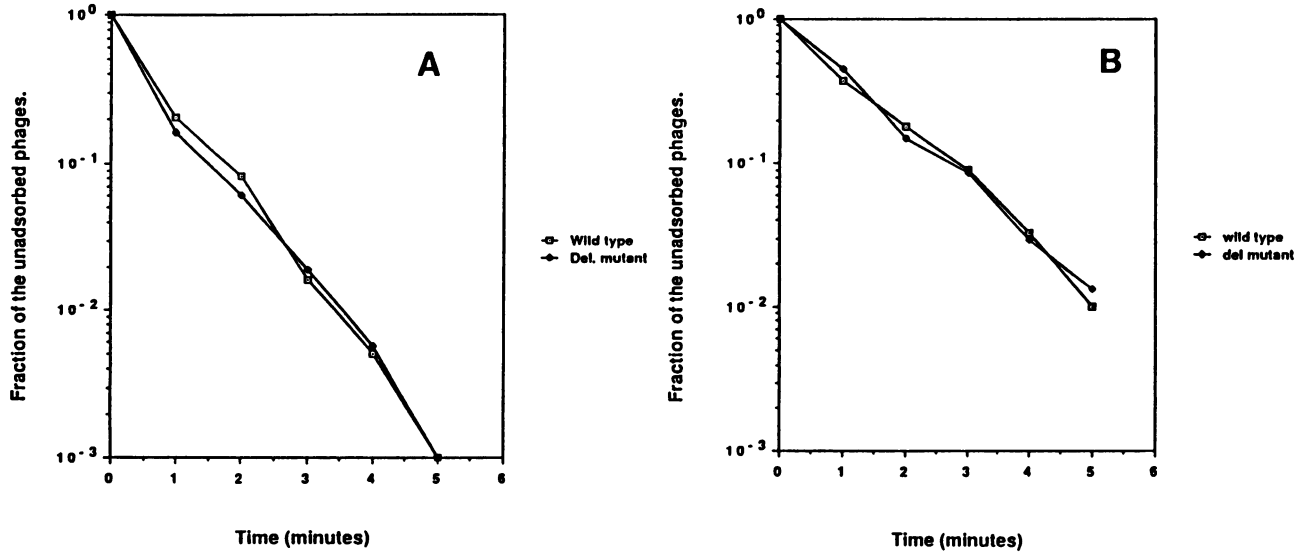

FIG. 11. Adsorption of T4 phage to its host. T4D or *del7* mutant phages were added to an *E. coli* cell culture, grown to a density of 2×10^8 cells per ml, at a multiplicity of 1 or 10. Every minute after addition of the phages, 100 μl of the cell culture was withdrawn and *E. coli* cells were inactivated by chloroform treatment. The unadsorbed phages remaining in the supernatant were diluted and plated in duplicate. Panels A and B show phage adsorption kinetics to *E. coli* B cells at multiplicities of 1 and 10, respectively.

TABLE 1. Phage burst sizes[a]

Phage strain	*E. coli* strain	Phage yield, PFU/cell
T4D	B	527
T4 r1589	B	171
T4 *del7*	B	10
T4D	pPS2/ED8689	109
T4 r1589	pPS2/ED8689	121
T4 *del7*	pPS2/ED8689	51

[a] Phage yields were determined under lysis-inhibited conditions previously described (24). The burst sizes on *E. coli* B are averages of five independent determinations, and the other burst sizes result from triplicate determinations.

properties of the T4 particle (3, 8, 9, 16). Second, both proteins are apparently bound in situ to the viral folate, as shown by cross-linking studies (25). Since the viral folate is present in amounts as high as 12 molecules per virion (L. M. Kozloff, personal communication), the proteins may be present in T4D at stoichiometric or near-stoichiometric levels. This is suggested also by inspection of gel electrophoretograms of purified T4 baseplates (6, 25, 26).

While the immunological evidence supporting a structural role for these enzymes must be reinterpreted, other lines of evidence remain intact. The poor growth of the deletion mutants in wild-type strains of *E. coli* suggests that the metabolic or structural roles played by these proteins, while not essential for phage viability, are quantitatively significant. Now that the deletions in *del7* and *del9* have been characterized and now that the purified gene products are available in quantity from cloned *td* and *frd* genes, we have a good opportunity to determine what those roles might be.

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ADDENDUM IN PROOF

In addition to burst sizes, *del7* has a plating efficiency about half that of T4D.

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