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Defective Concatemer Formation in Cells Infected with Deoxyribonucleic Acid-Delay Mutants of Bacteriophage T4[1]

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Nonpermissive host cells infected with phage T4 mutants in genes 52, 39, and 58 through 61 are shown to form short intracellular single-stranded deoxyribonucleic acid in contrast to wild-type infected cells, which form dimers and trimers of T4 genome size.

Many of the genes concerned with phage T4 deoxyribonucleic acid (DNA) synthesis have been studied, and their products are well characterized. However, the model for T4 DNA replication is not yet clear. Of possible help in clarifying this area should be studies on conditional lethal mutants blocked in the initial replication process. In the following, replicating intermediates formed by DNA-delay (DD) mutants in genes 52, 39, and 58 through 61 (2), known to initiate DNA synthesis at the normal time but at a reduced rate (13), were analyzed.

Escherichia coli strains B and CR 63 were obtained from F. W. Stahl. T4Dtd8BX4 was obtained from I. Tessman. T4Dam$^+$ and T4D DNA-delayed mutants amH17 (gene 52); amN116 (gene 39); amHL627 (genes 58 through 61; HL627 and E219 are believed to be in the same cistron [13]); and amE219 were obtained from F. W. Stahl. amE219 was found to contain a mutation to acridine resistance and was genetically purified by back-crossing to wild type.

In the absence of several early phage functions, a DNA-delay phenotype is obtained (2, 10, 13). The following experiments were undertaken with a view to determining the rate of synthesis under restrictive conditions, and the results of a typical experiment are shown in Fig. 1. It is seen that DNA synthesis (evaluated as ^{3}H-thymidine incorporation) starts around 6 min in each strain tested (13). However, the rate of synthesis declines 7 to 10 min after infection with DD mutants and reaches a minimum at 12 to 15 min. At that time, the control infection with wild-type phage shows 8 times more label incorporated under the experimental conditions. These results are probably not due to expansion of the deoxyribonucleotide pools in mutant-infected cells, since delayed DNA synthesis was also observed by the diphenylamine technique (2, 13). Stability of the DNA synthesized between 6 and 8 min was established in pulse-chase experiments indicating that the decrease in thymidine incorporation is not due to enhanced degradation. At later times during infection, an increase in the rate of synthesis can be observed. It is thus clearly seen that the function of genes 52, 39, and 58 through 61 is required for a normal rate of DNA synthesis during the first 15 min after infection.

It has been found (3, 4, 8) that, during later stages of infection by bacteriophage T4, long single strands are produced. These structures are of considerable interest because of the clues they may provide to the mode of DNA replication and to the efficiency of recombination under conditions of delayed DNA synthesis. To this end, the length of newly replicated DNA, formed from 6 to 12 min after infecton, was determined in alkaline sucrose gradients (9). The sedimentation profiles of the DNA synthesized after infection with T4td8 which served as wild type, or with one of the amber mutants, are shown in Fig. 2. Part A represents the profile obtained for td8 under standard conditions (3), and it is clearly seen that a considerable fraction (58%) of the DNA sedimented faster than mature phage DNA. The DNA synthesized by H17 (gene 52), N116 (gene 39), HL627 or E219 (genes 58 through 61; Fig. 2B, C, E, D) was present mainly as fragments smaller than mature T4 DNA molecules. Table 1 represents an attempt to summarize these findings. It is apparent that mutants H17 and N116 behave quite differently from E219 and HL627 in that the size distribution of DNA

[1] These results are part of a thesis submitted by Y. Naot in partial fulfillment of the requirements for the D.Sc. degree at the Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel.

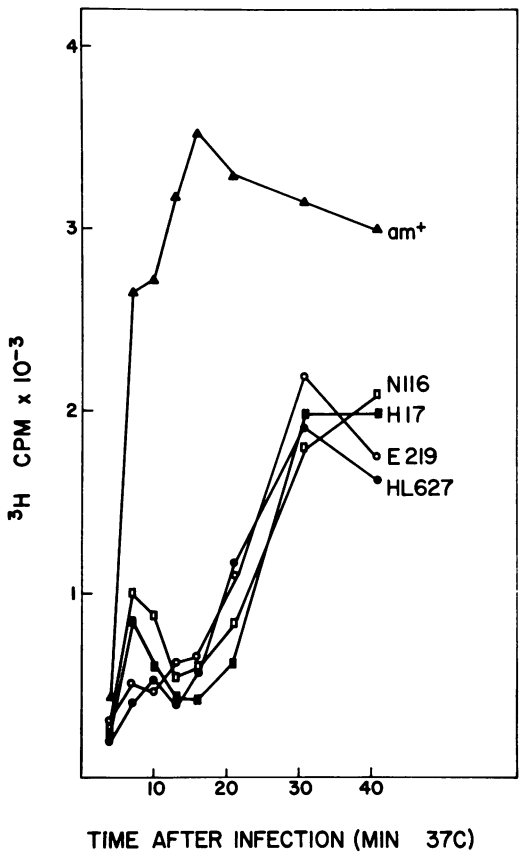

FIG. 1. *Rate of ^{3}H-thymidine incorporation in E. coli B infected with T4 DNA-delayed mutants. E. coli B was infected (multiplicity of infection = 5) with (▲) wild-type or with (■) H17 (gene 52), (□) N116 (gene 39), (●) HL627 (gene 58), (○) E219 (gene 61) at 26 C for 2 min. At zero time the infected culture was diluted to 2 × 10^8/ml in warm tris(hydroxymethyl)-aminomethane (Tris)-glucose medium supplemented with deoxyadenosine (250 μg/ml) at 37 C. At different times after infection, 1-ml samples were added to 0.2 ml of thymidine-methyl-^{3}H, 5 μCi/ml. After 2 min, the reaction was arrested with 2 ml of cold 10% trichloroacetic acid containing 1.8 mg of cold thymidine per ml. The precipitates were collected on membrane filters (Sartorius; 25 mm; 0.45 μm) and counted as described previously (9). The time of each point is the middle of the 2-min period.*

strands of the former is considerably shorter than the latter. Thus, it seems that, although genes 52, 39, and 58 through 61 are required for formation of long single chains, they do not all control the same function.

Having found no mutant newly synthesized DNA sedimenting more rapidly than mature-sized T4 DNA in the previous experiments, we tried to prevent endonucleolytic breakdown of concatemers by admixture of chloramphenicol known to inhibit late protein synthesis which includes nucleases (5). Accordingly, ^{3}H-thymidine (10 μCi/ml) was added at 6 min followed by chloramphenicol (Abic, Ramat Gan, Israel; 30 μg/ml) at 10 min after infection, (at which time it is known to permit appearance of fast-sedimenting DNA; references 4, 8), and the cells were further incubated up to 25 min. As much as 62% of the newly replicated DNA could be recovered in fractions sedimenting faster than mature T4 DNA in td8 and H17 infections (Fig. 3A and B). Suppression of the DNA-delay phenotype was found in H17-infected cells under experimental conditions (Naot and Shalitin, *manuscript in preparation*). By constrast, no long single strands were found in the cases of N116, E219, or HL627 under experimental conditions (Fig. 3C, D and E). In fact, chain length increased only slightly upon addition of chloramphenicol to E219- and HL627-infected cells, whereas in the case of N116 the increase was 2.5-fold (Table 1). Moreover, no effect on ^{3}H-thymidine incorporation is found if the drug is added 10 min post-infection with N116, HL627, and E219 (data not shown). In contrast, Bolle et al. (1) reported that addition of chloramphenicol (8 to 10 min at 30 C) restores N116 infected cells to a more normal time course of DNA synthesis.

If long single strands are formed through recombination only when 20 to 50 phage-equivalent units of DNA are synthesized (8), one can argue that the necessary amount of DNA is not accumulated in mutant infection. However, when chloramphenicol is added at 10 min after infection with

TABLE 1. *Molecular length of newly replicated DNA formed under restrictive growth conditions*

Mutant	Gene	Phage-equivalent units of length (L)[a]	
		Without chloramphenicol	With chloramphenicol
td8	td	1 to 3	1 to 4
H17	52	0.06	1 to 4
N116	39	0.09	0.23
HL627	58–61	0.40; 0.23	0.35
E219	58–61	0.40; 0.23	0.35

[a] Mean value obtained from sedimentation patterns in alkaline sucrose gradients represented in Fig. 2 and 3. Length (L) of concatemeric DNA calculated from the position of reference T4 DNA as previously described (9). When cut, the number of cuts (λ) per phage-equivalent unit of DNA was according to Litwin, Shahn and Kozinski, (7). Molecular length calculated from $1/\lambda + 1$.

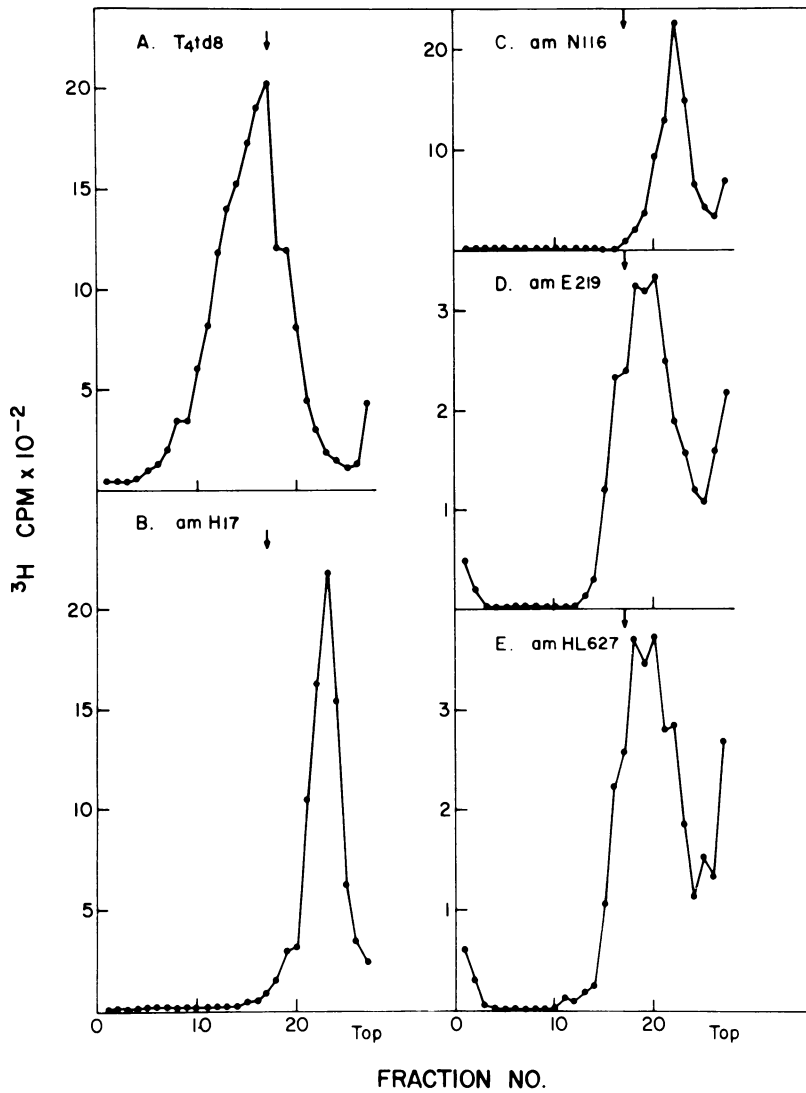

FIG. 2. *Concatemer formation in E. coli B cells infected with DNA-delayed mutants. E. coli B cells were infected as in legend to Fig. 1. Intracellular newly synthesized DNA was labeled with 10 μCi of ^{3}H-thymidine/ml at 6 min after infection. Samples were withdrawn at 12 min and poured onto cold KCN at a final concentration of 0.05 M. After fivefold concentration in Tris-glucose medium in $MgSO_4$ (2.5×10^{-3} M), an equal volume of ice-cold lysing solution, containing 0.01 M KCN, 0.1 M ethylenediaminetetraacetic acid in 0.1 M Tris, pH-8, and sodium dodecyl sulfate to a final concentration of 0.2%, was added. The lysates were kept overnight in the cold. (Under these conditions, no material sedimenting faster than reference DNA in alkaline sucrose gradients can be detected at early times after infection.) Alkaline sucrose gradient sedimentation was carried out as previously described (9). Centrifugation was performed in a Spinco model L-2 ultracentrifuge at 4 C in a SW50 rotor for 90 min at 32,000 rev/min. The arrows indicate the position obtained for reference ^{14}C-T4 DNA released from whole phage on top of the gradient. A, T4td8 (serving as wild-type); ^{3}H input, 5.85×10^4 counts/min. B, H17 (gene 52); ^{3}H input, 1.45×10^4 counts/min. C, N116 (gene 39); ^{3}H input, 1.13×10^4 counts/min. D, E219 (gene 61); ^{3}H input, 4.2×10^3 counts/min. E, HL627 (genes 58 through 61); ^{3}H input, 5.6×10^3 counts/min. Recoveries were about 90 to 100%.*

N116 and the cells are further incubated for a total of 45 min, no long single strands are found (Table 2) even though the amount of DNA synthesized in N116 infection (as judged by ^{3}H-thymidine incorporation) is at least the same as when concatemers are first formed during wild-type infection. This indicates that inhibition of N116 long-strand formation is due to defective

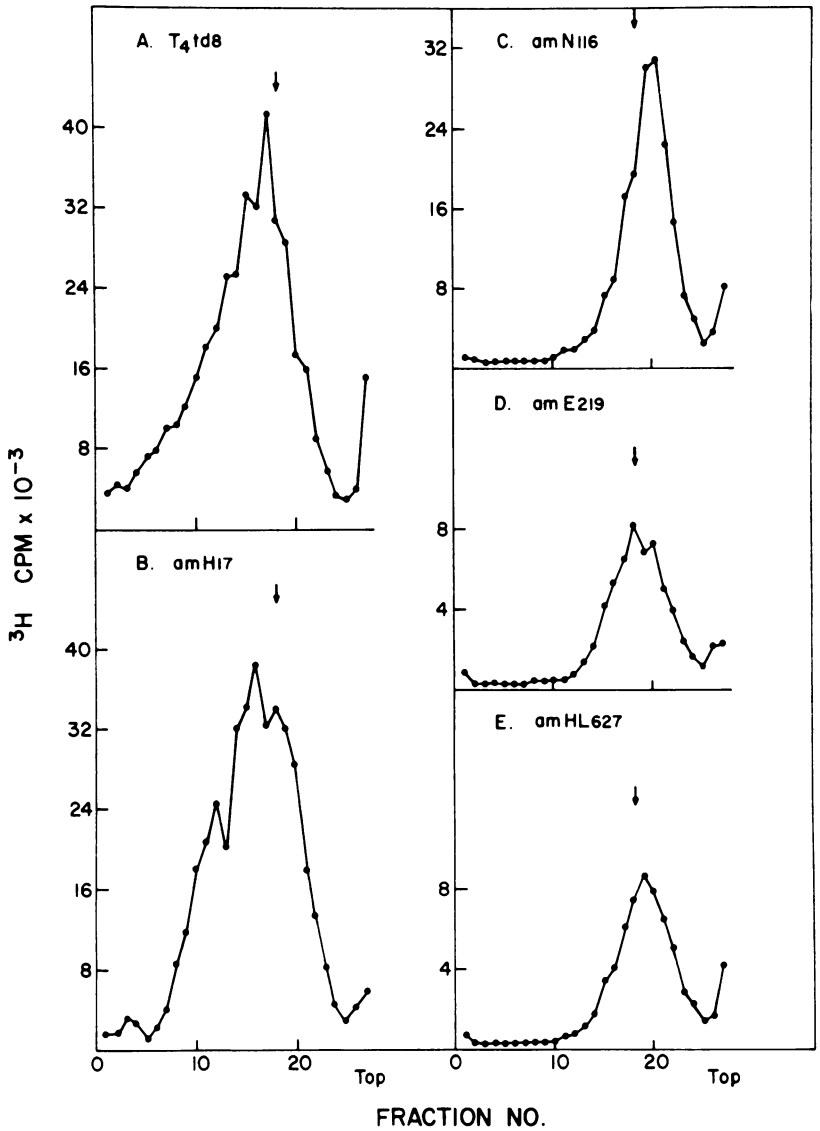

FIG. 3. *Effect of chloramphenicol on intracellular concatemer formation in E. coli B infected with DNA-delayed mutants. Intracellular DNA was labeled with ^{3}H-thymidine (10 μCi/ml) from 6 to 25 min after infection. Chloramphenicol (30 μg/ml) was added at the 10th min. Preparation of lysates and sedimentation through alkaline sucrose gradients as in legend to Fig. 2. The arrows indicate the position obtained for reference T4 single-stranded DNA. A, T4td8; ^{3}H input, 3.3 × 10^5 counts/min. B, H17 (gene 52); ^{3}H input, 1.7 × 10^5 counts/min. C, N116 (gene 39); ^{3}H input, 1.5 × 10^5 counts/min. D, E219 (gene 61); ^{3}H input, 3.1 × 10^4 counts/min. E, HL627 (gene 58–61); ^{3}H input, 3.5 × 10^4 counts/min.*

molecular recombination (8), rather than to shortage of DNA.

In view of the possible role of genes 52, 39, and 58 through 61 in T4 recombination, we assumed that some might code for nucleases involved in the degradation of *E. coli* DNA to deoxyribonucleotides. Previous work has shown that nuclease-defective mutants, such as gene 46 exonuclease (11), exonuclease A (J. F. Koerner and H. R. Warner, Fed. Proc., p. 1263, 1971), and endonuclease II (6; 12), produce no progeny phage in hyroxyurea-treated *E. coli* B. Accordingly, the effect of hydroxyurea (grade A, Calbiochem) on phage production by *E. coli* B cells

infected with DD mutants was tested, with results shown in Table 3. It is clearly seen that phage production in N116- and H17-infected cells is sensitive to hydroxyurea, indicating that the products of genes 39 and 52 play some role in the degradation of *E. coli* DNA to deoxyribonucleotides. The size of *E. coli* DNA remaining after infection with H17 will be shown in a forthcoming paper.

TABLE 2. *Amount of intracellular phage DNA versus molecular length (L)*

Time after infection[a] (min at 37 C)	T4Dam+		N116	
	Phage-equivalent units of DNA[b] per infective center	(L)[c]	Phage-equivalent units of DNA[b] per infective center	(L)[c]
12	34.4	1 to 3	9.0	0.09
25	85.6	1 to 4	25.0	0.23
30	102.4	1 to 4	39.0	0.35
45	148.0	1 to 4	85.0	0.35

[a] ^{3}H-thymidine added at 6 min, chloramphenicol at 10 min; experiment terminated at the time indicated in the table.

[b] The phage-equivalent unit of DNA is the amount of ^{3}H per infective phage particle, obtained in a highly purified preparation of wild-type or N116 phage grown in the same ^{3}H-labeled medium.

[c] Phage-equivalent units of length (L) calculated as described in footnote to Table 1.

TABLE 3. *Effect of hydroxyurea (HU) on phage production by DNA-delay mutants*[a]

Phage	Phage/infective center		+HU/−HU
	+HU	−HU	
T4Dam+	19.8	148	0.13
N116 (gene 39)	4.0	111	~0.03
H17 (gene 52)	3.0	112	~0.03
HL627 (gene 58–61	23.9	87.8	0.27
E219 (gene 58–61)	14.2	113	0.12

[a] *Escherichia coli* B at about 2×10^9 cells per ml was infected in KCN 0.002 M at 37 C at a multiplicity of 3 to 5. Antiserum was added to K = 3. HU was added at zero time to a final concentration of 0.01 M. Phage production was measured at 60 min at 37 C.

Functional blocks due to amber mutations in T4 genes 52, 39, and 58 through 61 may bring about a clear-cut deviation from the normal sedimentation pattern of intracellular phage DNA: (i) formation of single-stranded DNA fragments smaller than T4 genomes, whereas wild-type phage forms dimers and trimers of genome size; (ii) accumulation of normal-like fast-sedimenting DNA on admixture of chloramphenicol to H17-infected cells, whereas in the other mutants tested the drug had little effect on strand length.

The role of gene 52 in T4 DNA synthesis will be discussed in a forthcoming paper.

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