

# Amber Mutants of Bacteriophages T3 and T7 Defective in Phage-directed Deoxyribonucleic Acid Synthesis

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Amber mutants of the related phages T3 and T7 were isolated and tested for their ability to restore—as the wild type does—thymidine incorporation in ultraviolet (UV)-irradiated, UV-sensitive, nonpermissive host bacteria (*Escherichia coli* B<sub>s-1</sub>). Most amber mutants had this ability. However, in both T3 and T7, mutants unable to promote thymidine incorporation under these conditions were found and classified into two well-defined complementation groups: T3DO-A and T3DO-B, T7DO-A and T7DO-B. Infection of B<sub>s-1</sub> cells with representatives of groups DO-A had the following characteristics: (i) phage-directed uridine uptake in UV-irradiated cells was reduced to less than 20% of normal; (ii) breakdown of host deoxyribonucleic acid (DNA) was delayed and incomplete; (iii) no serum-blocking antigens appeared; (iv) no cell lysis occurred; (v) the ability to exclude the heterologous wild type was impaired. Amber mutants of the DO-B groups, infecting B<sub>s-1</sub>, were able to: (i) promote an efficient phage-directed uridine uptake in UV-irradiated cells; (ii) bring about rapid breakdown of host DNA; (iii) synthesize serum-blocking antigens; (iv) lyse the host cells, generally after the normal latent period; (v) exclude efficiently the heterologous wild type. Although physiological similarities between the respective DO-A mutants or DO-B mutants of T3 and T7 were evident, no physiological cross-complementation occurred, and genetic crosses gave no evidence of genetic homologies between groups of T3 and T7.

Coliphages T3 and T7 are closely related. They are morphologically identical (34), they cross-react serologically (1, 19), and their deoxyribonucleic acids (DNA) can be hybridized efficiently, by melting and annealing (29). Genetic recombination between T3 and T7 has also been reported (19). Notwithstanding their similarities, upon simultaneous mixed infection of *Escherichia coli* with these two phages, mutual exclusion occurs; i.e., in more than 90% of the host cells, only T3, or only T7, is produced (19, 20). The reasons for this are unknown, but so far we have ruled out mutual interference with adsorption and injection (*unpublished data*). To gather more information relevant to an understanding of the exclusion mechanism, we investigated more precisely the similarities and differences between T3 and T7, as far as the functions necessary for phage-directed DNA synthesis are concerned. The approach was to isolate amber mutants of these phages which are defective in phage-directed DNA synthesis, to characterize these mutants genetically and physiologically, and to investigate their behavior in regard to the phenomenon of

mutual exclusion. An abstract of part of this work has been published (*Bacteriol. Proc.*, p. 154, 1967).

## MATERIALS AND METHODS

**Bacteria.** Our permissive host, used for obtaining all lysates, was *E. coli* BBw/1. We derived it by two mutational steps from an *E. coli* BB strain which is permissive for certain amber mutants and was kindly sent to us from R. S. Edgar's laboratory. *E. coli* BBw/1 is more suitable as an indicator strain for color mutants (19) of T3 and T7 and is resistant to phage T1. As nonpermissive hosts, either *E. coli* B/5 or *E. coli* B<sub>s-1</sub> were used; the former also stems from R. S. Edgar's laboratory, the latter—an ultraviolet-sensitive strain (30)—is from W. Harm's collection. *E. coli* K-12 *thy<sup>-</sup> str<sup>s</sup> T<sub>DNA</sub><sup>s</sup>*, a mutant in which DNA synthesis is temperature-sensitive, was isolated by F. Bonhoeffer (2) and kindly given to us by H. Schuster.

**Phage.** The origin and characteristics of our reference types of T3 and T7, here called T3 $am^+$  and T7 $am^+$ , have been previously reported (18).

**Isolation of amber mutants.** Wild-type lysates grown on *E. coli* B/5 were treated with nitrous acid, hydroxylamine, or 2-aminopurine; in general, procedures as outlined by Edgar and Lielausis (11) or

Freese (16) were followed. The mutagen-treated lysates were grown for one cycle in BBw/1 to eliminate mutational heterozygotes. Phage from these secondary lysates were allowed to adsorb for 5 to 10 min to concentrated cultures of BBw/1 at multiplicities of less than  $10^{-2}$ , and were diluted into indicator mixtures containing BBw/1 and B/5 in the proportions of 1:3 (for T3) or 1:1 (for T7). Platings were made on dried color agar plates (4) by use of the spreading method. Amber mutants were recognized by their small and irregular plaques. They were picked and plated on BBw/1. Two or three random plaques grown on this host were then retested for the amber character by plating on BBw/1, on B/5, and on mixed indicator (BBw/1 plus B/5). If the mutants showed low levels of leakiness and low reversion frequencies, high-titer lysates in BBw/1 were made from a single plaque. The letter prefixes A, H, and N were used, respectively, to designate amber mutants isolated from lysates treated with aminopurine, hydroxylamine, and nitrous acid.

*Spot tests.* Complementation studies were carried out by spot-testing all amber mutants (15). A lysate to be tested was diluted, mixed with a culture of B/5, and plated by spreading. Immediately after the agar surface had dried, drops of diluted lysates of other amber mutants were placed on the same plate, which was incubated overnight. The tests were performed with different concentrations of phage, ranging from  $10^7$  to  $10^9$  particles/ml. Two amber mutants were considered to belong to different complementation groups if one spot in at least one concentration showed complete lysis, while the selfing controls at the same concentrations clearly showed no lysis.

*Growth conditions.* M9 medium supplemented with Casamino Acids (0.5%) was used (30); in crosses, nutrient broth was used. Unless otherwise stated, all experiments were performed at 37 C, with exponentially growing bacteria (about  $3 \times 10^8$  cells/ml), on a rotatory shaker.

*Ultraviolet (UV) irradiation.* Samples (10 ml) of a  $B_{s-1}$  culture were placed in a petri dish and exposed under continuous shaking to doses of 1,200 or 8,000 ergs/mm<sup>2</sup> of UV from a 15-w General Electric germicidal lamp. The lower or higher doses were given, respectively, when thymidine or uridine incorporation was measured. The cells were incubated for about 10 min to bring bacterial DNA (30) or DNA and ribonucleic acid (RNA) synthesis to a halt. <sup>3</sup>H-labeled thymidine or uridine, and phage, were then added. Burst size and latent period of wild-type phages were not influenced by irradiation of the host with the lower UV dose, but the higher dose markedly reduced the phage-producing capacity of the cells.

*Conditions of infection.* Phage was generally added at a multiplicity of at least 10 particles per cell. This assured infection of about 90% of the cells by at least one phage particle within 1 min, and of virtually all cells after 3 min. This was measured by viable cell counts in samples treated with antiphage serum. When mixed infections with T3 and T7 were performed, T3 was added at a multiplicity three times higher than T7, to compensate for the lower adsorption rate of T3.

*Thymidine or uridine incorporation.* When indicated,

1  $\mu$ c of <sup>3</sup>H-thymidine or 0.05  $\mu$ c of <sup>3</sup>H-uridine (Schwarz Bio Research Inc., Orangeburg, N.Y.) was added per ml of bacterial culture. As suggested by Boyce and Setlow (3), 250  $\mu$ g (per ml) of deoxyadenosine was added for improved thymidine incorporation. At intervals, 1-ml samples were chilled with equal volumes of ice-cold 10% trichloroacetic acid. The samples were filtered and washed with 5% trichloroacetic acid. The radioactivity retained on the membrane filters was measured in a Beckman scintillation counter. Incorporation of thymidine or uridine into acid-insoluble materials was considered to be a measure of DNA synthesis or RNA synthesis, respectively (30).

*Phage crosses.* A sample (0.5 ml) of an exponentially growing culture of permissive host bacteria ( $3 \times 10^8$  cells/ml) was added to an equal volume of a mixture of two different amber mutants ( $5 \times 10^8$  particles of each type, per ml). The phage mixture contained also 4 mM potassium cyanide. Five minutes was allowed for adsorption. The multiplicity of infection was about 10 particles of each parental phage. The infected cells were diluted into broth containing specific antiphage serum ( $K \approx 2$ ), and 2 mM potassium cyanide. Again after 5 min, the cells were further diluted, by a factor of  $10^{-4}$ , and allowed to lyse spontaneously. Platings were made on BBw/1 and on B/5. Since the frequency of back-mutants was negligible, phages forming plaques on B/5 were considered to be wild-type recombinants. Their percentage relative to the counts on BBw/1 was doubled (to account for double-amber recombinants), and then plotted on the linkage map.

*Other measurements.* DNA concentrations were measured by the method of Burton, as described by Epstein et al. (15). Cell densities were checked turbidimetrically with a Klett-Summerson colorimeter. Serum-blocking antigen was evaluated by the method of DeMars (6).

## RESULTS

*Identification of amber mutants defective in phage-directed DNA synthesis.* First, the 200-odd independently isolated amber mutants of T3, and about 150 of T7, were classified into complementation groups by spot-testing. Thus, 18 and 20 such groups were individualized, respectively, in T3 and T7. To identify those blocked in a function required for phage DNA synthesis, one or more mutants of each group were tested for their ability to restore thymidine incorporation upon infection of UV-irradiated  $B_{s-1}$  cells. [In these cells, no bacterial DNA synthesis takes place (30), and phage-directed DNA synthesis can be clearly distinguished.] For this purpose, <sup>3</sup>H-thymidine was added to a  $B_{s-1}$  culture which had been irradiated about 10 min earlier. Samples of this culture were inoculated with phage and further incubated for 25 min; radioactivity in the acid-insoluble fraction was then measured.  $T7am^+$  determined the incorporation of more than twice as much thymidine as did  $T3am^+$  (Table 1). Five of the T3

TABLE 1. Phage-directed incorporation of  $^3\text{H}$ -thymidine in UV-irradiated *Escherichia coli*  $B_{s-1}$ <sup>a</sup>

T3			T7		
Spot-test group	Phage	Thymidine incorporated	Spot-test group	Phage	Thymidine incorporated
	No phage	425		No phage	499
	<i>am</i> <sup>+</sup>	3,229		<i>am</i> <sup>+</sup>	7,929
1	<i>am</i> N19	388	1	<i>am</i> H280	437
2	<i>am</i> H5	432	2	<i>am</i> H30	431
3	<i>am</i> H13	488	3	<i>am</i> H131	514
4	<i>am</i> N22	336	4	<i>am</i> A11	7,566
5	<i>am</i> N104	465	5	<i>am</i> A21	8,970
6	<i>am</i> A47	3,095	6	<i>am</i> A24	6,609
7	<i>am</i> A59	1,188	7	<i>am</i> H5	4,286
8	<i>am</i> A69	1,145	8	<i>am</i> H6	7,252
9	<i>am</i> H4	3,923	9	<i>am</i> H10	7,366
10	<i>am</i> H26	2,127	10	<i>am</i> H11	8,567
11	<i>am</i> H34	2,524	11	<i>am</i> H13	3,508
12	<i>am</i> H42	3,486	12	<i>am</i> H20	6,767
13	<i>am</i> H44	5,277	13	<i>am</i> H51	7,322
14	<i>am</i> H63	2,200	14	<i>am</i> H71	6,202
15	<i>am</i> H84	3,682	15	<i>am</i> H80	7,161
16	<i>am</i> N76	1,458	16	<i>am</i> H90	9,272
17	<i>am</i> N90	3,087	17	<i>am</i> H108	8,823
18	<i>am</i> N158	3,140	18	<i>am</i> H111	7,991
			19	<i>am</i> H127	7,525
			20	<i>am</i> N72	6,175

<sup>a</sup> An exponentially growing culture of *E. coli*  $B_{s-1}$  ( $3 \times 10^8$  cells/ml) was given 1,200 ergs/mm<sup>2</sup> of UV and incubated for 5 min, to bring bacterial DNA synthesis to a halt.  $^3\text{H}$ -Thymidine (1  $\mu\text{C}$ /ml) was added; immediately afterwards, samples of the culture were infected with phage (multiplicity of infection, 10) and incubated at 37 C for 25 min. Radioactivity of the fraction insoluble in cold 5% trichloroacetic acid was then measured, and is expressed as counts per minute per milliliter of culture.

amber spot-test groups and three of the T7 amber groups showed a complete inability to promote phage-directed thymidine incorporation. [Following the nomenclature of Epstein et al. (15), such mutants will henceforth be referred to as DO mutants (DNA-zero).] Levels of incorporation to varying degrees lower than normal were also found with several group representatives, but T3*am*H44 consistently showed incorporation levels 50 to 100% higher than wild type. Such abnormal levels were characteristic of certain whole groups, not only of some of its representative mutants.

Some mutants of T3 and T7 with such anomalous patterns were investigated in respect to the time course of thymidine incorporation after infection of UV-irradiated nonpermissive bacteria. T3 wild-type and several amber mutants of

T3 determined a sharp onset of thymidine incorporation 5 to 6 min after infection, but different final levels were reached (Fig. 1A). The previously identified DO mutants did not promote

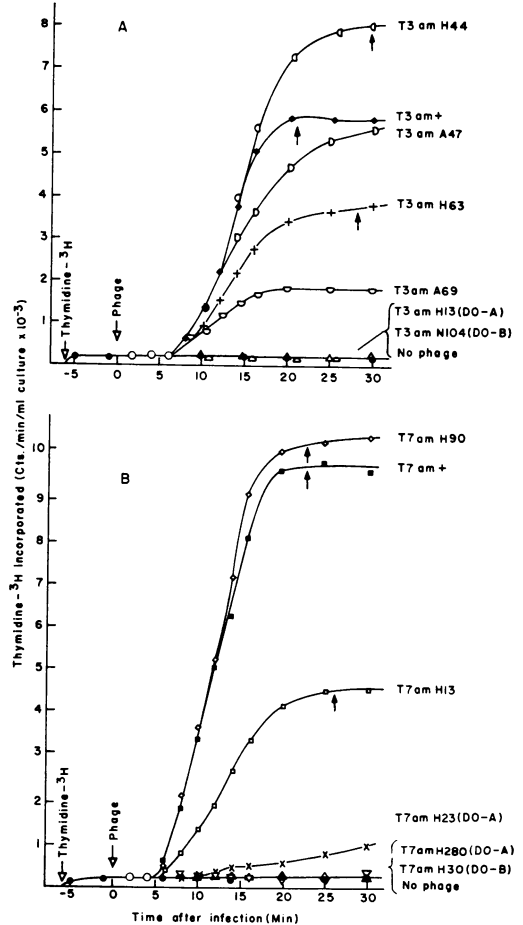


FIG. 1. Time course of thymidine incorporation after infection of UV-irradiated *Escherichia coli*  $B_{s-1}$  with T3*am*<sup>+</sup> or some amber mutants of T3 (Fig. 1A), and with T7*am*<sup>+</sup> or some amber mutants of T7 (Fig. 1B). An exponentially growing culture of *E. coli*  $B_{s-1}$  ( $3 \times 10^8$  cells/ml) was given a UV dose of 1,200 ergs/mm<sup>2</sup> and incubated for 5 min at 37 C.  $^3\text{H}$ -Thymidine (1  $\mu\text{C}$ /ml) was added and the incubation was continued for 6 min. Samples of this culture were then infected with phage (multiplicity of infection, 10). At intervals, 1-ml samples were withdrawn for measuring radioactivity in the fraction insoluble in cold 5% trichloroacetic acid. The open circles cover points referring to all curves. Black arrows indicate time of visible lysis of a culture. The designations DO-A and DO-B refer to complementation groups, as defined in the text. T7*am*H23 is one among several "leaky" mutants of group DO-A. Other mutants of this group, such as T7*am*H280, did not promote thymidine incorporation above the background of the uninfected host.

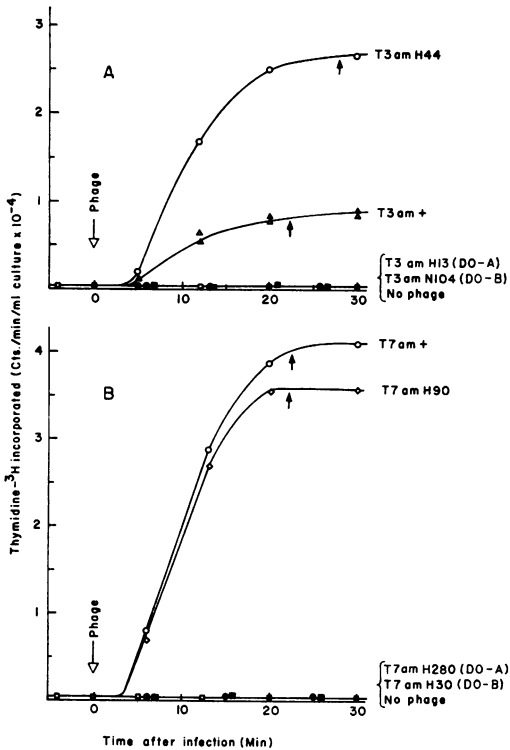


FIG. 2. Time course of thymidine incorporation after infection at 41 C, of *Escherichia coli* K-12  $T_{DNA}^S$  with T3am<sup>+</sup> or amber mutants of T3 (Fig. 2A), and with T7am<sup>+</sup> or amber mutants of T7 (Fig. 2B). Bacteria were grown at 30 C to a concentration of about  $5 \times 10^7$  cells/ml, then warmed to 41 C and further incubated for 15 min. Labeled thymidine (1  $\mu$ Ci/ml) was added. A few minutes later, phage was added. At intervals, 1-ml samples were withdrawn for measuring radioactivity in the fraction insoluble in cold 5% trichloroacetic acid. Multiplicity of infection was 10 phages per cell, except for the curve represented by black triangles (multiplicity of 40). Black arrows indicate visible lysis of the culture.

incorporation of thymidine at any time. After infection with T7am<sup>+</sup>, thymidine incorporation was initiated after a similar lag period of 5 min; however, compared to T3am<sup>+</sup> infection, it proceeded at about twice the rate (Fig. 1B). Most DNA-synthesizing amber mutants of T7 were like the wild type in this respect. But, as in T3, some of them incorporated thymidine at a lower rate than did the wild type, although this incorporation started after the normal lag. In T7, spot-test group 1 contained, besides unambiguous DO mutants, some "leaky" mutants, such as T7amH23, which showed a residual thymidine incorporation (up to 10% of the normal rate). The shape of all the curves was not affected by changing the multiplicity of infection within the tested range of 3 to 30 particles per cell.

Initiation of thymidine incorporation in irradiated  $B_{s-1}$  cells was shown to depend on phage-directed protein synthesis, as judged by the fact that it was totally inhibited by the addition, together with the phage inoculum, of 30  $\mu$ g (per ml) of chloramphenicol.

**Infection of *E. coli* K-12  $T_{DNA}^S$  by T3, T7, and their DO-amber mutants.** Besides knocking out host DNA synthesis by UV, another procedure for distinguishing T3- or T7-directed DNA synthesis was used. It consisted of infecting *E. coli* K-12  $T_{DNA}^S$ , a nonpermissive strain in which DNA synthesis is temperature-sensitive (2), at a temperature of 41 C. Under these conditions, DO-amber mutants were not able to promote thymidine incorporation, although the wild-type phages and other amber mutants were able to do so (Fig. 2). It is evident, however, that this ability was greatly reduced in T3am<sup>+</sup> as compared to T7am<sup>+</sup>. Under these conditions, the rate of thymidine incorporation by T3am<sup>+</sup> was only 20% that of T7am<sup>+</sup>, as compared to 50% when UV-irradiated  $B_{s-1}$  cells were used as hosts.

**Patterns of complementation and genetic recombination of DO-amber mutants.** Table 2 shows the classification of our DO-mutants into spot-test groups. The allocation of some mutants to a specific group was ambiguous, and three other criteria were used to clarify this issue. These were (i) determination of average burst sizes per cell when different pairs of DO-amber mutants were crossed in the nonpermissive host B/5, (ii) measurements of thymidine incorporation under conditions of mixed infection of UV-irradiated  $B_{s-1}$  cells, and (iii) genetic mapping of DO mutants.

Tables 3 and 4 show for T3 and T7, respectively, the average burst sizes per nonpermissive, and as a control, per permissive cell, after mixed infection with two DO mutants. Under nonpermissive conditions, the burst sizes in crosses of two representatives of the same spot-test group were generally in the range of the background of a self cross; crosses between mutants of different spot-test groups yielded bursts varying from 0.2 to 80 particles. Bursts in the permissive host ranged from 40 to 130 particles. Burst sizes smaller than five particles per nonpermissive cell posed the question of whether functional complementation had occurred or not, since they were below the burst sizes obtained with other pairs of mutants by a factor of 10 or more.

Measurements of thymidine incorporation under conditions of mixed infection of UV-irradiated  $B_{s-1}$  cells were of further help in establishing the degree of functional complementation between DO-amber mutants. The poorly "complementing" combinations of spot-test group representatives of T3, i.e., combinations with T3amN19, T3amH5, and T3amH13 on one hand,

TABLE 2. Allocation of DO-amber mutants to complementation groups

Phage	Complementation group	Spot-test group	Mutant stocks
T3	T3DO-A	1	<i>amN19</i> <sup>a</sup> ; <i>amA34</i> (?) <sup>b</sup> ; <i>amN69</i>
		2	<i>amH5</i> ; <i>amA34</i> (?); <i>amN108</i>
		3	<i>amH13</i> ; <i>amH79</i>
	T3DO-B	4	<i>amN22</i> ; <i>amA31</i> ; <i>amH84</i> ; <i>amN18</i>
		5	<i>amH104</i> ; <i>amA31</i> (?); <i>amH15</i> ; <i>amN18</i> (?)
T7	T7DO-A	1	<i>amH280</i> ; <i>amH23</i> ; <i>amH31</i> ; <i>amH205</i> ; <i>amH264</i> ; <i>amH283</i> ; <i>amH335</i> ; <i>amH358</i>
	T7DO-B	2	<i>amH30</i> ; <i>amA3</i> ; <i>amH70</i> ; <i>amH102</i> ; <i>amH114</i> ; <i>amN71</i>
		3	<i>amH131</i> ; <i>amH70</i> (?)

<sup>a</sup> The first mutant in each row is the arbitrarily chosen reference type against which all other mutants were tested.

<sup>b</sup> Interrogation marks refer to dubious cases.

TABLE 3. Average burst sizes per nonpermissive or permissive cell after mixed infection with two DO-amber mutants of T3

Complementation group	Spot-test group	Phage	DO-A			DO-B	
			Spot-test group 1 ( <i>amN19</i> )	Spot-test group 2 ( <i>amH5</i> )	Spot-test group 3 ( <i>amH13</i> )	Spot-test group 4 ( <i>amN22</i> )	Spot-test group 5 ( <i>amN104</i> )
DO-A	1	<i>amN19</i>	<0.05 (99) <sup>a</sup>	— <sup>b</sup>	—	—	—
		<i>amN69</i>	0.1 (108)	0.8 (91)	0.3 (128)	27 (73)	20 (95)
		<i>amA34</i>	<0.05 (77)	0.3 (121)	0.2 (105)	18 (78)	36 (126)
DO-A	2	<i>amH5</i>	0.3 (103)	0.1 (59)	—	—	—
		<i>amH13</i>	0.2 (73)	0.5 (70)	<0.05 (99)	—	—
DO-B	4	<i>amN22</i>	21 (109)	27 (61)	19 (86)	<0.05 (128)	—
		<i>amN18</i>	20 (82)	25 (46)	14 (58)	<0.05 (103)	0.6 (66)
		<i>amA31</i>	23 (67)	38 (74)	23 (85)	<0.05 (77)	0.3 (70)
	5	<i>amN104</i>	25 (82)	29 (113)	20 (73)	1.7 (60)	<0.05 (59)
		<i>amH15</i>	28 (64)	36 (58)	27 (109)	0.2 (57)	0.1 (93)

<sup>a</sup> Burst sizes below 0.05 could not be evaluated because of the background of unadsorbed input phages which had survived antiserum. Crosses were performed in *Escherichia coli* B/5 (nonpermissive) or *E. coli* BBw/1 (permissive). Burst sizes were obtained by dividing phage titers after spontaneous lysis by the titer of plaque-forming units before lysis. The burst sizes per permissive cell, infected in identical conditions, are given in parentheses.

<sup>b</sup> See same cross in another box.

and T3*amN22* with T3*amN104* on the other, brought about no appreciable increase of thymidine-incorporating ability. In other pairwise combinations, the spot-test group representatives of T3 restored thymidine incorporation to values 30 to 85% of normal (Table 5). In T7, values of 70 to 95% were found with representatives of spot-test group 1 on one side and groups 2 or 3 on the other. Between groups 2 and 3, complementation was less than 10% of normal (Table 6).

For mapping purposes, crosses between DO mutants were performed in the permissive host BBw/1, and the progeny were plated on permissive and on nonpermissive (B/5) indicator bacteria. Particles growing on B/5 were considered

*am*<sup>+</sup> recombinants. The map distances shown in Fig. 3 refer to twice the percentage of *am*<sup>+</sup> recombinants relative to the total progeny, so as to account for the reciprocal (double-amber) recombinants. In T3, representatives of spot-test groups 1, 2, and 3 mapped in the same segment of the T3 genome, extending over 7 map units (1 unit defined as 1% recombination between two markers); representatives of groups 4 and 5 mapped in another segment, extending over 9 units. The distance between the closest representatives of each segment was about 9 units, and no representative of other spot-test groups have as yet been found to lie in between. In T7, a somewhat similar situation was found. Representatives of

TABLE 4. Average burst sizes per nonpermissive or permissive cell after infection with two DO-amber mutants of T7<sup>a</sup>

Complementation group	Spot-test group	Phage	DO-A	DO-B	
			Spot-test group 1 (amH280)	Spot-test group 2 (amH30)	Spot-test group 3 (amH131)
DO-A	1	amH280	<0.05 (77)	—	—
		amH23	<0.05 (51)	35 (42)	45 (70)
		amH205	0.1 (67)	29 (58)	36 (65)
DO-B	2	amH30	23 (53)	<0.05 (54)	
		amN71	43 (59)	0.1 (45)	4.6 (64)
	3	amH114	30 (45)	0.2 (38)	3.8 (52)
		amH131	27 (66)	3.5 (64)	0.2 (50)

<sup>a</sup> Procedures as in Table 3.

TABLE 5. Complementation pattern between DO-amber mutants of T3 as measured by <sup>3</sup>H-thymidine incorporation in UV-irradiated *Escherichia coli* B<sub>s</sub>-1<sup>a</sup>

Group	Phage	am <sup>+</sup>	DO-A			DO-B	
			amN19	amH5	amH13	amN22	amN104
Wild type	am <sup>+</sup>	4,385	4,591	4,752	4,372	4,921	4,396
DO-A	amN19 amH5 amH13		303	261 189	161	1,570	1,966
					343	2,814	3,439
					228	1,464	3,077
DO-B	amN22 amN104				250	187	
						104	

<sup>a</sup> The procedure was as outlined in Table 1, except that, when two phages were used, the multiplicity of infection was 5 of each. The results are expressed as counts per minute per milliliter of culture. When no phage was added, the activity was 220 counts per min per ml.

spot test group 1 mapped in one segment, whereas representatives of groups 2 and 3 mapped in another, separated from the former by 14 map units (Fig. 3). The relative positions of these groups of T3 or T7 with respect to representatives of other spot-test groups have not yet been established unambiguously because of loose linkage relationships.

The data derived from these mapping experiments, as well as from burst size measurements and stimulation of <sup>3</sup>H-thymidine incorporation in nonpermissive conditions, led us to establish two well-defined complementation groups which are necessary for phage-directed DNA synthesis to occur in both phages, T3 and T7. As a working hypothesis, the poorly "complementing" spot-test groups were considered to belong to the same functional unit, since their representatives mapped at distant sites within one map segment and the low levels of "complementation" might be attributable to the formation of wild-type recombinants, rather than to functional comple-

mentation. The groups established were called T3DO-A and T3DO-B, T7DO-A and T7DO-B (without implying genetic homology between homologously named groups of T3 and T7). Group T3DO-A comprises spot-test groups 1, 2, and 3, and spot-test groups 4 and 5 constitute group T3DO-B. Groups T7DO-A and T7DO-B comprise spot-test group 1 and spot-test groups 2 and 3, respectively (Table 2).

In the following, representative mutants of these groups will be further characterized in regard to their physiology upon infecting nonpermissive cells.

*Cell lysis and synthesis of serum-blocking antigens.* All mutants of groups T3DO-A and T7DO-A were unable to lyse the nonpermissive host and did not promote synthesis of serum-blocking antigens. All mutants of groups T3DO-B and T7DO-B did lyse the nonpermissive host (although with some mutants, lysis was delayed); they promoted synthesis of serum-blocking antigens within the

TABLE 6. Complementation pattern between DO-amber mutants of T7 as measured by thymidine incorporation in UV-irradiated *Escherichia coli* B<sub>s-1</sub><sup>a</sup>

Group	Phage	am <sup>+</sup>	DO-A			DO-B		
			amH280	amH30	amH131			
Wild type	am <sup>+</sup>	8,273	8,154	7,052			8,957	
DO-A	amH280		226	5,867			7,823	
DO-B	amH30 amH131			215		776 263		

<sup>a</sup> Procedure and definitions as outlined in Table 5. The background activity (no phage added) was 308.

nonpermissive cells, although in diminished amounts compared to the wild types.

**Postinfection breakdown of host DNA.** Since most of the DNA of T3 and T7 is built up from components of the host (21), an extensive breakdown of host DNA must precede or accompany phage-directed DNA synthesis. This breakdown was detected by measuring the amount of acid-insoluble radioactive material within labeled host cells, at various times after infection with phage. Nonpermissive host cells were grown in the presence of <sup>3</sup>H-thymidine and centrifuged when their concentration reached 3 × 10<sup>8</sup> cells/ml. The pellet was rinsed, and then suspended in unlabeled medium at the original temperature (37 C) and cell concentration. The resuspended culture was divided in samples which were infected either with wild-type or with a DO mutant of either T3 or T7. The wild-type phages brought about a sharp, albeit limited, solubilization of previously incorporated thymidine, starting about 5 min after infection (Fig. 4). Solubilization leveled off after a few more minutes, and a slow reincorporation of solubilized material took place until the cells lysed. We interpret these kinetics as a composite of two phenomena, reflecting the breakdown of host DNA, which provides a pool of components for the subsequent synthesis of phage DNA.

The DO-amber mutants showed a different behavior. With representatives of groups T3DO-B and T7DO-B, the onset of solubilization of previously incorporated thymidine occurred at the same time as with the wild types, but it leveled off much later. Only about 40 and 15%, respectively, of the host label remained acid-precipitable after infection by these amber mutants of T3 or T7; no reincorporation of

solubilized material occurred in these cases. With representatives of groups T3DO-A and T7DO-A, the onset of solubilization of the host label was delayed and the rate of solubilization was less than one-third as compared to the homologous representatives of group DO-B (Fig. 4). In all cases, breakdown of host DNA after phage infection could be prevented by chloramphenicol (30 μg/ml), added together with the phage.

When similar experiments were done, but without removing labeled thymidine before infection, the results obtained allowed additional information about the behavior of DO mutants (Fig. 5). Again, the representatives of groups T3DO-B and T7DO-B brought about an easily observable solubilization of previously incorporated thymidine after a lag of about 5 min. Up to this time, however, incorporation of thymidine by the host continued. This continued thymidine incorporation in the (unirradiated) host was more pronounced after infection with the representatives of groups T3DO-A and T7DO-A (in which breakdown of host DNA was delayed). With T3amH13, representing group DO-A, the reduced phage-induced breakdown, as observed in the experiment of Fig. 4, was completely masked by the continuing incorporation of thymidine. We consider the residual incorporation of thymidine by unirradiated B<sub>s-1</sub> cells, after infection by DO-amber mutants, as representing synthesis of host-specific DNA. This view was taken considering that thymidine incorporation by this UV-sensi-

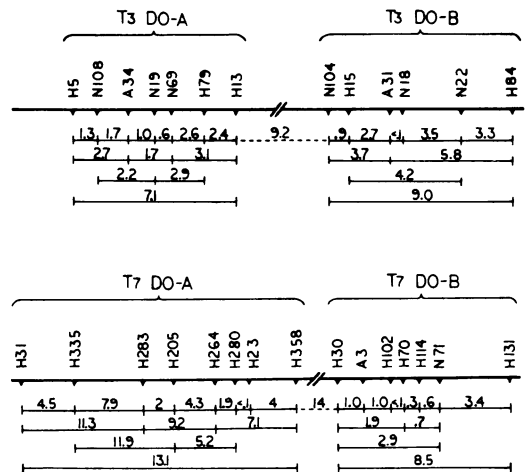


FIG. 3. Linkage map of some DO-amber mutants of T3 and of T7. Distances are given as twice the percentages of plaques on the nonpermissive indicator relative to plaques on the permissive one. The values are averages of two to four crosses.

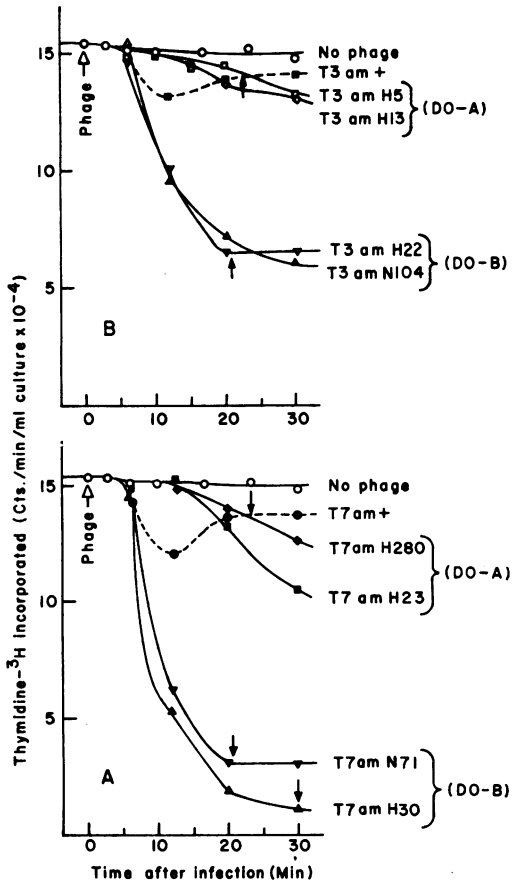


FIG. 4. Breakdown of host DNA after infection with DO mutants of T3 (Fig. 4A) or T7 (Fig. 4B). Nonpermissive cells were grown in the presence of tritiated thymidine for several generations, then washed and suspended in unlabeled medium. A few minutes later, phage was added at a multiplicity of 10 particles per cell, and the cells were further incubated at 37 C. At intervals, 1-ml samples were added to equal amounts of ice-cold 10% trichloroacetic acid. Acid-insoluble radioactivity was plotted. Black arrows indicate the time of visible lysis.

tive host could be totally prevented with a small UV dose prior to infection by DO-amber mutants.

The data referring to thymidine incorporation after phage infection were corroborated by chemical determinations by Burton's method (15) of net DNA content of phage-infected cells (Fig. 6).

**Phage-directed uridine uptake.** The procedure for these experiments was similar to those aimed at characterizing the DO mutants by their inability to promote phage-directed thymidine uptake (Fig. 1), except that the UV-sensitive host cells were given a higher dose of UV (8,000 ergs/mm<sup>2</sup>) and, instead of 1  $\mu$ C of <sup>3</sup>H-thymidine,

0.05  $\mu$ C of <sup>3</sup>H-uridine was added per ml of culture (Fig. 7). Uridine incorporation by the irradiated cells was resumed 2 to 3 min after the addition of wild-type phage and leveled off at about 10 min. Contrary to phage-directed thymidine uptake (which was totally inhibited), the rate of uridine incorporation was reduced by only about 30% by the addition of chloramphenicol (30  $\mu$ g/ml). Mutants of groups T3DO-B and T7DO-B showed an incorporation rate quite similar to the wild types, although the level of incorporation was somewhat lower (Fig. 7). Uridine uptake determined by representatives of groups T3DO-A and T7DO-A, however, was sharply reduced, as compared to the wild types and other DO-amber mutants. In T7amH23, the most efficiently incorporating representative of group T7DO-A, uridine uptake was limited to 20% that of wild type; in group T3DO-A, the maximum was 5% that of wild type (Fig. 7). In all cases, no appreci-

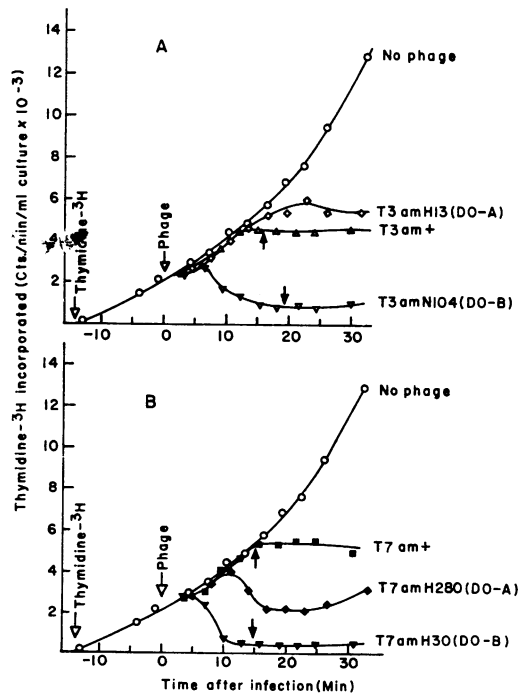


FIG. 5. Time course of thymidine incorporation after infection of exponentially growing *Escherichia coli* B<sub>8-1</sub> cells with T3am<sup>+</sup> or some amber mutants of T3 (Fig. 5A) and with T7am<sup>+</sup> or some amber mutants of T7 (Fig. 5B). <sup>3</sup>H-thymidine (1  $\mu$ C) was added when the bacteria had reached a concentration of  $2 \times 10^8$  cells/ml. After 14 min, samples of this culture were infected with phage (multiplicity of infection, 10). At intervals, 1-ml samples were withdrawn for measuring radioactivity in the acid-insoluble fraction. Black arrows indicate the time of visible lysis.



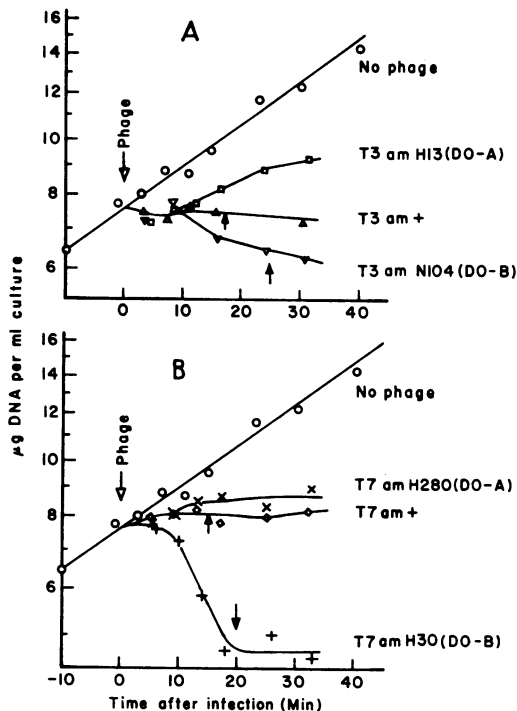


FIG. 6. Net DNA content of cultures of *Escherichia coli*  $B_{s-1}$ , at various times after infection with the wild type or some amber mutants of T3 (Fig. 6A) or T7 (Fig. 6B). Samples of an exponentially growing culture were infected with different mutant strains of T3 or T7 at a multiplicity of about 5 phages per cell and further incubated (37 C). At intervals, 8-ml samples were chilled with 2 ml of 70% perchloric acid at 0 C, and their DNA content was evaluated by the method of Burton. Black arrows indicate the time of visible lysis of an infected culture.

able effect of multiplicity of infection was detected.

**Lack of heterologous complementation between DO-amber mutants.** In previous experiments we had observed that some amber mutants of T3 and T7 were capable of cross-complementing each other, at least to some extent, in spite of their mutual exclusion from the progeny of a mixedly infected cell (*to be published*). In heterologous crosses between DO-amber mutants of T3 and T7, using a nonpermissive host, we found no such cross-complementation in any combination of T3 and T7 mutants. Absence of cross-complementation was also found at the level of DNA synthesis by measuring thymidine incorporation after pairwise infection of irradiated restrictive cells with one T3 mutant and one T7 mutant (Table 7).

**Mutual exclusion between T3 and T7, as affected by DO-amber mutations.** To test how DO-amber

mutations affect the exclusion ability (19, 20) of the corresponding genomes, representatives of the groups DO-A and DO-B of T3 and of T7 were crossed with the heterologous wild types in the nonpermissive host. It was reasoned that mixed infection should be abortive in the fraction of cells in which the wild type was excluded by heterologous amber mutant. (This holds true only in the absence

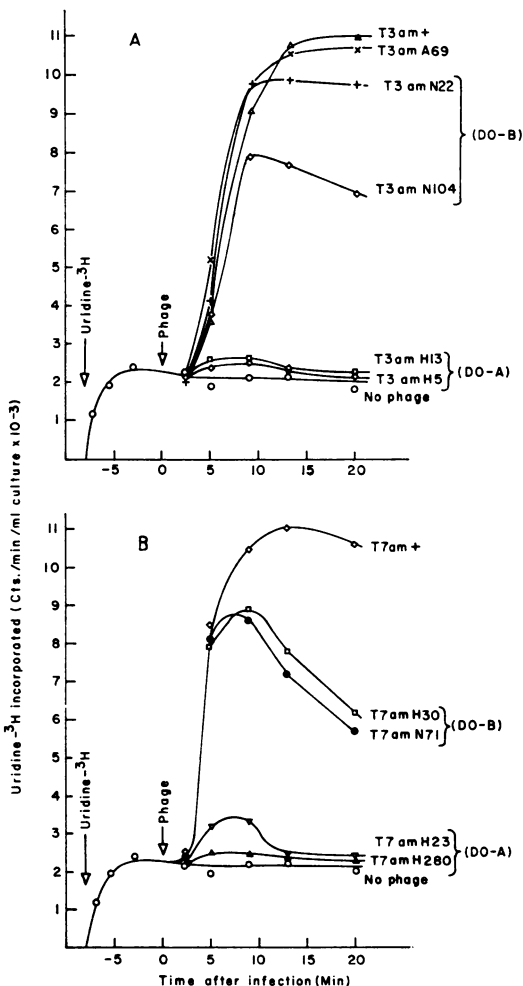


FIG. 7. Time course of phage-directed uridine incorporation after infection of UV-irradiated *Escherichia coli*  $B_{s-1}$  with  $T3am^+$  or some amber mutants of T3 (Fig. 7A), and with  $T7am^+$  or some amber mutants of T7 (Fig. 7B). An exponentially growing culture ( $3 \times 10^8$  cells/ml) was given a UV dose of 8,000 ergs/mm<sup>2</sup> and incubated for 10 min at 37 C. <sup>3</sup>H-uridine (0.05 µc/ml) was added and the incubation was continued for 8 min. Samples of this culture were then infected with phage (multiplicity of infection, 10). At intervals, 1-ml samples were withdrawn for measuring radioactivity in the fraction insoluble in cold 5% trichloroacetic acid.

TABLE 7. Lack of heterologous complementation between DO-amber mutants of T3 and T7, as measured by thymidine incorporation in UV-irradiated *Escherichia coli* B<sub>s</sub>-1<sup>a</sup>

Group	Phage	Blank	T7 am <sup>+</sup>	DO-A		DO-B	
				T7 amH280	T7 amH30	T7 amH131	
Wild type	Blank	270	7,934	336	206	216	
	T3 am <sup>+</sup>	4,686	5,935	4,260	1,513	1,685	
DO-A	T3 amN19	205	8,707	288	303	241	
	T3 amH5	301	8,819	271	350	245	
	T3 amH13	128	7,324	240	216	191	
DO-B	T3 amN22	305	4,978	185	266	218	
	T3 amN104	112	3,582	212	258	241	

<sup>a</sup> The procedure was as outlined in Table 1, except that T3 was added at a multiplicity of 15, and T7 at a multiplicity of 5, to compensate for differences in adsorption rate. Under these conditions, the offspring of a culture infected with T3 am<sup>+</sup> and T7 am<sup>+</sup> was about 50% of each type.

of heterologous complementation. Platings on permissive indicator after lysis of the mixedly infected cells indeed revealed the absence of the amber parental type in the progeny.) On the other hand, if the function impaired by an amber mutation was essential to the exclusion mechanism, the growth of a wild-type phage would not be affected by the presence of such a heterologous amber mutant. Tables 8 and 9 show data pertinent to this type of cross. The relative input multiplicities of the parental types were so chosen (20) as to allow about 80 to 90% of the mixedly infected cells to exclude the heterologous wild type, if the same cross was performed as a control in the permissive host. The mixedly infected cells were plated before lysis, and the wild type-producing infective centers were counted. (On both permissive and nonpermissive indicators, similar numbers of wild type-producing infective centers were counted.) Control experiments, in which cells were infected by the wild type only and plated similarly, indicated the number of wild type-producing cells in the absence of exclusion. The difference between the plaque counts in these experiments indicated the number of cells in which the wild type had been excluded by the co-infecting heterologous amber mutant. Representatives of groups DO-B in both phages excluded the heterologous wild-type phage nearly to the same extent in the nonpermissive and in the permissive hosts. The exclusion capacity of representatives of groups DO-A, however, was severely impaired in the nonpermissive host.

A corresponding observation was made at the level of phage-directed thymidine incorporation after mixed infection of nonpermissive cells with DO-amber mutants and heterologous wild type:

representatives of the DO-A groups were incapable of appreciably reducing the amount of thymidine incorporated by co-infecting heterologous wild-type phage (Table 7). This indicates that no exclusion of wild-type functions had occurred. On the other hand, representatives of the DO-B groups, upon mixed infection in the

TABLE 8. Ability of T3 to exclude T7 am<sup>+</sup>, as affected by DO-amber mutations<sup>a</sup>

T3 stock used as one parental type		T7-producing infective centers (× 10 <sup>-7</sup> ) per ml of adsorption mixture	
Group	Phage	Cross in permissive host	Cross in non-permissive host
Wild type	T3am <sup>+</sup>	1.5 (12%)	1.7 (11%)
DO-A	T3amH5	2.3 (18%)	12.6 (84%)
	T3amH13	1.9 (15%)	9.8 (65%)
DO-B	T3amN17	1.1 (8.5%)	1.8 (12%)
	T3amN104	2.1 (16%)	3.1 (21%)

<sup>a</sup> Heterologous crosses, with samples of the same mixtures of parental phages, were done both in *Escherichia coli* BBw/1 (permissive host) and *E. coli* B/5 (nonpermissive host). Multiplicities of infection were about 30 for T3 and about 3 for T7. The infected bacteria were plated on B/5 before lysis. The infective centers producing T7am<sup>+</sup> were counted and compared to the number of infective centers produced in control experiments in which the cells had been infected with T7am<sup>+</sup> only. The concentration of infective centers per ml of adsorption mixture in the control experiments was 1.3 × 10<sup>8</sup> for B/5 and 1.5 × 10<sup>8</sup> for BBw/1; these are the 100% values, to which the percentages in parentheses refer.

TABLE 9. Ability of T7 to exclude T3am<sup>+</sup>, as affected by DO-amber mutations<sup>a</sup>

T7 stock used as one parental type		T3-producing infective centers (X 10 <sup>-7</sup> ) per ml of adsorption mixture	
Group	Phage	Cross in permissive host	Cross in non-permissive host
Wild type	T7am <sup>+</sup>	2.2 (17%)	3.0 (20%)
DO-A	T7amH23	2.5 (19%)	10.8 (72%)
	T7amH280	1.3 (10%)	8.9 (59%)
DO-B	T7amH30	3.0 (23%)	4.5 (30%)
	T7amH131	1.8 (14%)	3.6 (24%)

<sup>a</sup> Multiplicities of infection were about 6 for T3 and about 20 for T7. See also legend to Table 8.

same condition, depressed thymidine incorporation by the heterologous wild type by 40 to 50% (T3am and T7am<sup>+</sup>) or by about 70% (T7am and T3am<sup>+</sup>).

#### DISCUSSION

It is most likely that reinitiation of thymidine incorporation 5 to 6 min after infection of UV-irradiated *E. coli* B<sub>s-1</sub> cultures with T3 or T7 is due to the synthesis of phage-specific DNA, since Swenson and Setlow (30) have shown, and we have confirmed, that in that strain bacterial DNA synthesis, as measured by thymidine incorporation, stops shortly after UV irradiation and cannot be resumed. It could be argued, however, that the infecting phage genome introduces genetic information for a repair mechanism which would allow host DNA synthesis to be restored. There is evidence that no such mechanism exists in either T3 or T7, since the UV sensitivity of these phages is considerably higher when plated on *E. coli* B<sub>s-1</sub> as compared to that on *E. coli* B. If these phages had an efficient repair mechanism of their own [as in T4, for instance (17)], survival of irradiated phage should be largely independent of the characteristics of the host. Besides, since a nearly normal burst of phage particles results from infection of B<sub>s-1</sub> cells irradiated with 1,200 ergs/mm<sup>2</sup> of UV, phage-specific DNA synthesis must obviously occur. Thus, although more direct evidence, such as hybridization data with phage-DNA, is still lacking, we interpret thymidine incorporation after infection of UV-irradiated *E. coli* B<sub>s-1</sub> cells as reflecting exclusively the synthesis of phage-specific DNA. In any event, however, the lack of thymidine incorporation after infection with DO-amber mutants of T3 or T7 can be attributed to amber-determined blocks

of specific gene functions related to DNA synthesis.

These functions were also required for phage-directed thymidine incorporation at 41 C, upon infection of *E. coli* K-12 T<sub>DNA</sub>, a bacterial strain which has been shown to have a temperature-sensitive DNA-synthesizing machinery (2). Under these conditions the wild types of T3 and T7 determined an efficient resumption of thymidine incorporation, followed by cell lysis and phage production, whereas DO mutants were totally inactive. These results again argue against a repair function of the DO genes.

The similarity of behavior of T3 and T7 with respect to phage-directed DNA synthesis was somewhat unexpected in view of the observations of Cox and Yanofsky (*personal communication*) that T3 clearly induces an increase in DNA polymerase activity after infection, whereas T7 does not, and that T7 is susceptible to the mutagenic effects of a genetically altered DNA polymerase of the host, and T3 is not. But the fact that in both phages, T3 and T7, genetic functions have here been identified which are necessary for phage-directed DNA synthesis is not in contradiction with the possibility of host DNA polymerases using one or both of these genomes as templates, since phage-specific functions might be necessary for making the phage genomes available to the host enzyme. In fact, only the single-stranded DNA of the small phages  $\Phi$ X174 and S13 is known to be used directly as template for some DNA synthesis by host enzymes (7, 31). (This results in the formation of the replicative form of these phages.) In phage  $\lambda$ , three well-defined cistrons (genes *N*, *O*, and *P*) are required for the initiation of phage-specific DNA-synthesis (9), besides a fourth recently identified genetic factor (14). Radding (26) showed that the *N* gene influences the formation of the  $\lambda$ -specific exonuclease, although it is not the structural gene for this enzyme (27). Nothing is known about the functions of genes *O* and *P*, but it has been shown that genes *N*, *O*, and *P* do not interfere with the formation of a circular  $\lambda$  DNA species from the linear infecting DNA (28). In T4, 12 genes necessary for phage-specific DNA synthesis have been reported (12); the functions of 3 of these genes have been identified (8, 32, 33).

Of 18 complementation groups in T3, as determined by spot tests, 5 turned out to be totally deficient in phage-directed DNA synthesis; of 20 such groups in T7, 3 showed this characteristic. However, upon testing pairwise combinations of group representatives of T3 or T7, only two clear-cut complementation groups could be found in these phages by the criterion of restoration of thymidine incorporation in UV-irradiated

$B_{s-1}$  cells. Burst-size measurements of mixedly infected nonpermissive cells, mapping experiments, and physiological data supported this latter finding. This confirmed the observation of Edgar et al. (10) that spot tests are more reliable when negative, since the formation of intracistronic wild-type recombinants can be sufficiently frequent to bring about a complete clearing of the spot. We thus divided our DO mutants of T3, as well as those of T7, into two complementation groups, DO-A and DO-B. The possibility cannot be excluded, however, that these groups consist of more than one cistron. Polarity effects, as described by Nakata and Stahl (23) for T4, could lower the level of complementation between adjacent cistrons.

The exact role of the DO functions in these phages is not known, but some physiological features of the infectious process by these DO mutants deserve some comment. DO-B mutants had the ability to initiate breakdown of the host DNA after the same time period as found upon wild-type infection, i.e., about 5 min, at 37 C. Within 10 min, DO-B mutants of T3 solubilized 60%, and those of T7 up to 90%, of the  $^3\text{H}$ -thymidine previously incorporated by the host (Fig. 4). In contrast, DO-A mutants were found to initiate breakdown later and at a much lower rate. In both cases, however, this breakdown was dependent on protein synthesis after infection, since it did not occur in the presence of chloramphenicol. This is not evidence, however, for the synthesis of phage-specific nucleases after infection, since the phage-induced intracellular alterations might have the effect of causing the bacterial DNA to become accessible to the already existing host nucleases. In fact, Pardee and Williams (25) reported that no appreciable increase in deoxyribonuclease activity occurs after T3 infection, contrary to what is observed with the T-even phages and with  $\lambda$  (24, 35).

A strikingly distinctive difference between DO-A and DO-B mutants is the fact that phage-directed uridine uptake (which was observable 2 min after infection of heavily UV-irradiated  $B_{s-1}$  cells) was not very markedly reduced in DO-B mutants; in DO-A mutants, only a maximum of 20% of the level of incorporation relative to the wild type was found. With some DO-A mutants, incorporation could hardly be distinguished from the background of the uninfected irradiated host. We have not yet worked out further details about the absence of phage-directed uridine uptake by DO-A amber mutants of T3 or T7, but present ideas about the physiological processes of phage infection suggest that it reflects interference of the amber lesion with synthesis of phage-specific messenger RNA. This could occur by several

mechanisms. For example, sequential transcription of operon-like structures could be prevented by an amber-determined lack of synthesis of a specific inducer. A more direct inhibitory action of the amber mutation could be envisaged if one considers the still controversial interdependence of transcription and translation (5, 13). It could also be thought that DO-A amber mutations affect the synthesis of a protein which is necessary for completing the injection of the phage genome, in analogy to what has been shown for phage T5 (22). However, this probably does not occur in the present case, since phage-directed uridine incorporation by the wild types was only slightly affected by the presence of chloramphenicol, denoting efficient injection in presence of the drug.

Another physiological distinction between different types of DO mutants is the fact that, in both T3 and T7, DO-A mutants do not lyse the nonpermissive cells and do not promote synthesis of serum-blocking antigens, whereas DO-B mutants lyse these cells and synthesize serum-blocking antigens. Thus, DO-B mutants show a behavior quite different from what is known for T4 (15) and  $\lambda$  (9), in which so-called "late functions," like cell lysis and synthesis of serum-blocking power, are not expressed if DNA synthesis is genetically impaired.

Since T3 and T7 are related phages, and their DO-A and DO-B mutants, respectively, have quite similar physiological characteristics, the question of the genetic homology between these mutant groups of T3 and of T7 arises. Genetic crosses have not been informative because no DO-amber mutations can as yet be crossed into a heterologous genome. Since functional cross-complementation has also not been observed, no indication of genetic homology between these physiologically similar functions is as yet available. We have, however, isolated several hybrids among amber mutants (in addition to DO mutants) of T3 and T7 *am*<sup>+</sup> which are genetically compatible with T7 (no exclusion) but carry the T3 amber site. Thus, the question of genetic homology will be further pursued. Whether non-complementation is due to the fact that the heterologous gene products cannot be utilized by the phage-producing machinery of either T3 or T7, or whether the mechanism of mutual exclusion prevents heterologous gene products from being formed, cannot yet be decided.

In regard to the mechanism of mutual exclusion, it seems quite clear that the DO-A function is required, whereas the influence of the DO-B function of T3 or of T7 is minimal or nonexistent. Thus, mutual exclusion could be thought of as a relatively early event, in spite of the fact that expression of the "early" *sam*<sup>+</sup> gene in T3 (18)

does not seem to be specifically inhibited by the exclusion mechanism. Exclusion might occur at a stage of phage development between the synthesis of "early" proteins and the onset of phage-directed DNA synthesis. Further work will be oriented towards evaluating the importance to the exclusion mechanism of other genetic functions, whose expression might be affected by nonexpression of the DO-A function.

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