The DNA-Delay Mutants of Bacteriophage T4

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Mutants of phage T4 defective in genes 39, 52, 58-61, and 60 (the DNA delay or DD genes) are characterized by a delay in phage DNA synthesis during infection of a nonpermissive *Escherichia coli* host. Amber (*am*) mutants defective in these genes yield burst sizes varying from 30 to 110 at 37 C in *E. coli* lacking an *am* suppressor. It was found that when DD *am* mutants are grown on a non-permissive host at 25 C, rather than at 37 C, phage yield is reduced on the average 61-fold. At 25 C incorporation of labeled thymidine into phage DNA is also reduced to 3 to 10% of wild-type levels. Mutants defective in the DD genes were found to promote increased recombination as well as increased base substitution and addition-deletion mutation. These observations indicate that the products of the DD genes are necessary for normal DNA synthesis. The multiplication of the DD *am* mutants on an *Su*⁻ host at 37 C is about 50-fold inhibited if prior to infection the host cells were grown at 25 C. This suggests that a compensating host function allows multiplication of DD *am* mutants at 37 C in the *Su*⁻ host, and that this function is active in cells grown at 37 C prior to infection, but is inactive when the prior growth is at 25 C. Further results are described which suggest that the products of genes 52, 60, and 39 as well as a host product interact with each other.

Mutants defective in the four bacteriophage T4 genes 39, 52, 60, and 58-61 have a similar phenotype characterized by a delay in phage DNA synthesis when they infect a nonpermissive *Escherichia coli* host. Mutants of gene 58-61 were originally misclassified into two complementation groups corresponding to genes 58 and 61. Subsequently, however, it was recognized (31) that these mutations belong to one cistron and we now designate this gene as 58-61. Mutants defective in these four genes are said to have a DNA-delay phenotype (11) and are referred to as DNA-delay or DD mutants. All DD *am* mutants tested are leaky. That is, whereas *am* mutants usually produce very low, if any, yield of viable phage in an *E. coli* host that lacks an *am* suppressor, the DD *am* mutants grow on cells lacking the suppressor and produce a substantial yield of progeny phage. The leakiness of these DD mutants has impeded efforts to determine their functional defect.

Evidence is presented here which indicates that a host product can compensate for the loss of DD gene function and that the DD mutants are defective in DNA synthesis. Evidence is also presented which indicates that the product of gene 39 interacts with a host product as well as with the products of genes 52 and 60. Our

observations, taken together with those of others, suggest that these products are necessary for normal DNA synthesis and that they interact with each other and a bacterial component probably at the membrane.

MATERIALS AND METHODS

Phage and bacterial strains. All T4 phage mutants used in this study came from the California Institute of Technology collection under the care of W. Wood. The designations of these mutants are indicated in the gene maps shown in Fig. 1. Three *E. coli* strains lacking an *am* suppressor S/6, 594, and B/5, as well as two strains containing an *am* suppressor, CR63 and 011', were used. *E. coli* 594 (4) and CR63 (1) were derived from *E. coli* K12, whereas S/6, B/5, and 011' were derived from *E. coli* B (11, 31).

Media. H-broth was used for growth and suspension of phage and bacteria. EHA bottom and top agar were used for plaque assays or for plating bacteria (25). M9 medium (6) was used for growth of infected cells in DNA-labeling experiments.

Preparation of phage stocks. The phage stocks used were clonally derived from single plaques. High titer phage stocks were prepared by a plate lysate method adapted from that described by Edgar (9). Phage lysates used for [³H]thymidine incorporation experiments were prepared on M9 plates or in M9 liquid medium without thymidine.

Preparation of bacterial plating cultures. Fresh log phase cultures of bacteria were used for phage assays. These were prepared by diluting a fresh overnight culture 1:1,000 in H-broth and growing the

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cells to 10^8 per ml. The cells were then centrifuged in the cold and resuspended in one-tenth of the original volume of H-broth.

Test of efficiency of plating. Efficiency of plating on a particular host strain was calculated as the percentage of the number of plaques obtained on that strain to the number formed on *E. coli* CR63 at 37 C when equal portions of phage were plated.

Preparation of bacteria as host for phage in liquid cultures. Host strain cultures were prepared from a 1:100 dilution of an overnight culture in H-broth and grown to 4×10^8 cells per ml.

Burst size determinations. Bacterial hosts used in burst size determinations were grown at the requisite temperature as described above. Just prior to phage infection, a portion of cells was plated to determine the titer of bacteria. Without any change of growth conditions, 0.02 ml of 0.05 M KCN was added to 0.5 ml of the bacteria. Then 0.5 ml of phage (at an MOI of about 10) were added. After 10 min at 37 or 40 C (or 15 min at 25 C) to allow for phage adsorption, the mixture was diluted 4×10^4 -fold into H-broth. This dilution of the KCN allows initiation of phage growth. A 1.0-ml portion of the diluted phage-cell mixture was then immediately transferred to 1.0 ml of H-medium plus chloroform and a sample was plated on *E. coli* CR63 at 37 C to determine the titer of unadsorbed phage. After 60 to 120 min, depending on growth temperature, chloroform was added to the growth cultures and the titer of progeny phage was determined by plaque assay with *E. coli* CR63. Phage burst size was calculated as the ratio of the phage titer to the titer of infected bacteria. Since the burst sizes of wild-type phage at 25 and 37 C did not differ significantly under the conditions of these experiments, there did not appear to be any differential loss of progeny due to the cyanide treatment at the two temperatures.

Temperature-shift experiments. The host bacteria were grown as described above at 25 C and when their concentration reached about 4×10^8 , 0.5 ml of the suspension was added to a tube containing 0.5 ml of phage (at a titer of 2×10^9) plus 0.02 ml of 0.05 M KCN at 37 C. After 10 min for adsorption, the culture was diluted at 37 C to allow growth and the procedure was followed as in the preceding section. A similar procedure was followed when infected cultures were shifted from 37 to 25 C.

Bacteriophage crosses. Phage crosses were performed at 37 C using *E. coli* CR63 as a host by the method described by Steinberg and Edgar (25). Total phage progeny was determined by plaque assay on *E. coli* CR63 and incubating these cultures at permissive temperatures, and recombinants were determined by plaque assay of progeny from phage crosses under nonpermissive conditions (*E. coli* S/6 at 25 C for *am* $\times$ *am* crosses, *E. coli* 594 λ at 25 C for *am* $\times$ *rII* crosses and *E. coli* S/6 at 45 C for *ts* $\times$ *ts* crosses).

Preparation of double mutants. *Am ts* double mutants were isolated by spotting individual plaques from the lysate of an *am* $\times$ *ts* cross under permissive conditions (*E. coli* CR63 at 25 C) and each of the nonpermissive conditions (*E. coli* CR63 at 45 C and *E. coli* S/6 at 25 C). *Am ts* double mutants would not grow on *E. coli* S/6 and produce no plaques or tiny

plaques on *E. coli* CR63 at 45 C. Individual plaques from the *am* $\times$ *ts* cross were touched with a toothpick and consecutively stabbed on plates containing *E. coli* S/6 and CR63 which were incubated under the conditions indicated. The presence of the *ts* mutation in most presumed *am ts* double mutants was confirmed by selecting *am*⁺ revertants of the double mutants on *E. coli* S/6 at 25 C and then testing these revertants for capacity to grow at the nonpermissive temperature, 45 C.

Am rII double mutants were constructed by testing individual plaques from the progeny of *am* $\times$ *rII* crosses made under permissive conditions (*E. coli* CR63, 37 C). The plaque assays were made on *E. coli* S/6 at 25 C and on *E. coli* K12-594 λ at 37 C (nonpermissive conditions for *am* and *rII* mutants, respectively) and *E. coli* CR63 at 37 C (permissive condition). The segregants with the double mutant phenotype were picked from the permissive indicator plates, suspended in H-broth, and after suitable dilutions again were tested for their double mutant phenotype by plating under permissive and nonpermissive conditions. The double mutant stocks were grown as described above.

Complementation tests. Mixed infections to test complementation between mutants were performed under nonpermissive or partially permissive conditions. *Am* $\times$ *am* mixed infections were performed using *E. coli* S/6 at 25 C. Total progeny was measured by plating on *E. coli* CR63 at 37 C.

DNA synthesis; measurement of incorporation of [methyl-³H]thymidine into acid-insoluble material. *E. coli* S/6 was grown in M9 medium at 25 C. When the bacterial concentration reached 4×10^8 , 0.5 ml of the culture was added to 0.5 ml of a suspension of phage (at a titer of about 2×10^9) supplemented with 0.034 μ g of [³H]thymidine per ml at a specific activity of 15 Ci/mM. Samples (0.1 ml) of the infected culture were withdrawn at regular intervals and immediately precipitated in 1.0 ml of ice cold 5% trichloroacetic acid containing 250 μ g of thymidine per ml. Each of the samples was repeatedly washed with the 5% trichloroacetic acid solution and filtered on a membrane filter (Millipore Corp.). The filters were dried and radioactivity of each was measured in a liquid scintillation counter using 4 g of BBOT per liter of toluene. BBOT was obtained from Packard Instrument Company, Downers Grove, Ill.

RESULTS

Effect of temperature on efficiency of plating of DD *am* mutants. In general, *am* mutants do not form plaques on *E. coli* S/6, the standard nonpermissive host for *am* mutants. In contrast, all *am* mutants defective in the four DD genes (39, 52, 60, and 58-61) are characteristically able to grow and form small plaques on *E. coli* S/6. The present investigation started with the observation, made jointly with M. Vallée in this laboratory, that the DD *am* mutants form plaques at 37 C or above but yield no plaques when plated at 25 C on *E. coli* S/6. The plating characteristics of DD *am* mutants were

FIG. 1 A and B

FIG. 1. Genetic maps of genes 39(a), 52(b), 58-61(c), and 60(d). The map distances shown were derived from two-factor crosses and are expressed in % recombination. Distances obtained by Parma and Snyder (21) are shown in parentheses. Using *rII* duplications, Parma (personal communication) determined the following order of mutations: *rII*, *amE429*, *amE300* and *amE416*, where the three *am* mutations are in gene 60. This corroborates the order of sites shown in (d). As indicated in the standard map of phage T4 (30), the direction of transcription of early function genes has so far always been found to be in a counterclockwise direction. The direction of transcription indicated by the arrows in (a) and (d) is the one obtained by assuming that the direction of transcription of genes 39 and 60 is the same as that of the other early genes. Superscripts accompanying map distances signify the number of individual measurements used in determining the indicated average value.

investigated by comparing their efficiency of plating on *E. coli* S/6 vs *E. coli* CR63 at 37 C. It was found that efficiency of plating was relatively reproducible for a given mutant in repeated determinations, but varied from mutant to mutant. The efficiency of plating of DD *am* mutants varied from as high as 70% to values below 1%.

A genetic map was constructed for each of the four DD genes using recombination percentages obtained in two-factor crosses (Fig. 1). The efficiency of plating of each mutant on *E. coli* S/6 was compared to its map position. Table 1 shows the efficiency of plating of representative mutants from the beginning, middle, and the end of genes 39, 52, and 60. This sam-

pling illustrates that the variation in efficiency of plating of the mutants did not show any striking correlation with the position of the mutants on the gene maps. Whereas in gene 60 the mutants with the highest efficiency of plating were in the beginning of the gene and those with the lowest efficiency of plating were at the end of the gene, genes 52 and 39 showed a more variable pattern. The data for gene 58-61 are not given since mutants in this gene were too few to determine whether a pattern was present.

To show that the efficiency of plating of a given *am* mutation is characteristic of that mutation and is relatively unaffected by subtle differences in the genetic backgrounds of the

FIG. 1 C and D

strains, crosses were carried out to wild-type phage and *am* segregants were examined for their efficiency of plating. The two most leaky gene 39 mutants (*amE158* and *amE556*) and the two least leaky ones (*amE480* and *amE604*) were crossed to wild-type, T4D⁺. Ten *am* segregants from each cross were plated out on *E. coli* S/6 and *E. coli* CR63 at 37 C to determine

their efficiency of plating. The results of this experiment are shown in Table 2. The standard deviation in each set of 10 is small and consistent with the range of experimental error. This result indicates that the degree of leakiness of a mutant is not significantly influenced by factors in its genetic background.

Comparison of burst size and efficiency of

TABLE 1. Efficiency of plating of representative *am* mutations in genes 39, 52, and 60^a

Mutant	Map position	Efficiency of plating
Gene 39 <i>am</i> NG457	0	27
<i>am</i> E672	0.46	69
<i>am</i> E26	7.3	20
<i>am</i> E480	8.6	10
<i>am</i> E556	13.8	70
<i>am</i> E839	15.0	10
Gene 52 <i>am</i> E670	0	23
<i>am</i> H17	8.8	5
<i>am</i> NG576	9.8	26
<i>am</i> E663	13.6	19
<i>am</i> E1111	14.9	53
Gene 60 <i>am</i> E416	0	62
<i>am</i> E412	0.21	55
<i>am</i> E1217	0.2	16
<i>am</i> E300	2.2	15
<i>am</i> HL626	3.2	12

^a Efficiency of plating estimates were calculated as the percentage of plaques on *E. coli* S/6 compared to *E. coli* CR63 at 37 C. The values given are the averages of two separate measurements. Map positions of these mutants are from Fig. 1 and were obtained by summing the percentage of recombination between neighboring mutant sites on the map.

plating of individual mutants. Efficiency of plating may be a measure of a different parameter than burst size, e.g., efficiency of plating might reflect the initiation of replication, whereas burst size would indicate what follows it in the final product. To determine whether the two measures correlate at a growth temperature of 37 C, burst sizes of two gene 39 *am* mutants giving a high efficiency of plating and two having a low one were measured (Table 3). No correlation between efficiency of plating and burst size was apparent. Although we are uncertain of the explanation for the lack of correlation, efficiency of plating depends both on burst size and latent period (the interval between infection and release of progeny virus after spontaneous lysis). Since the latent period is prolonged with the DD *am* mutants compared to wild type (31), it is possible that variation in this period is responsible for the lack of correlation.

Effect of host strain and temperature on burst size of DD mutant phage. Since the plating characteristics of DD *am* mutants are affected by temperature, it is worthwhile determining the temperature effect on burst size of the phage. Representative DD *am* mutants were grown on two different Su⁻ strains of *E. coli* and two different Su⁺ strains of *E. coli* at 25 and 37 C. When *E. coli* S/6, a Su⁻ strain, was used as a host there was an increase in burst size

from an average of 1.0 at 25 C to 61 at 37 C (Table 4 columns 1 and 2). Wild-type phage had reasonably high burst sizes at the two temperatures. A gene 32 *am* mutant, which does not have the DD phenotype, had burst sizes below 1 both at 25 and 37 C on *E. coli* S/6. A second Su⁻ strain of *E. coli*, 594, also yielded a lower burst size at 25 than at 37 C for the DD *am* mutants, but the differences were not as dramatic (Table 4, columns 3 and 4). The average burst size at 25 C was 42, whereas at 37 C it was 75. It appears that at 25 C *E. coli* 594 is not as restrictive to multiplication of DD *am* mutants as *E. coli* S/6.

TABLE 2. Efficiency of plating of the segregating *am* progeny from cross of selected gene 39 *am* mutants to T4D⁺^a

Segregant	<i>am</i> E158 × T4D ⁺	<i>am</i> E556 × T4D ⁺	<i>am</i> E480 × T4D ⁺	<i>am</i> E604 × T4D ⁺
1	89	78	8	17
2	90	80	8	18
3	90	81	17	23
4	71	80	19	20
5	85	78	13	11
6	86	90	21	14
7	85	86	20	18
8	68	94	9	16
9	65	70	19	12
10	69	68	15	19
Mean	80	81	15	17
Standard deviation	9	8	5	3

^a The *am* mutants were crossed to T4D⁺ as described in Materials and Methods. Progeny phage were plated on *E. coli* CR63 at 37 C. Individual plaques were picked, suspended in H-broth, and assayed on *E. coli* S/6 at 25 and 37 C, and on *E. coli* CR63 at 37 C. Only segregants which did not grow on *E. coli* S/6 at 25 C (i.e., the segregants with the DD *am* phenotype) were tested for their efficiency of plating on *E. coli* S/6 compared to *E. coli* CR63 at 37 C.

TABLE 3. Comparison of burst size and efficiency of plating of representative gene 39 *am* mutants at 37 C^a

Phage strain	Efficiency of plating on <i>E. coli</i> S/6	Burst size	
		<i>E. coli</i> S/6	<i>E. coli</i> CR63
<i>am</i> N116	9	104	130
<i>am</i> E480	10	77	95
<i>am</i> E158	70	85	115
<i>am</i> E556	75	55	75
T4D ⁺	100	146	136

^a All values represent an average of two or more determinations. Details of procedures for determining efficiency of plating and burst size appear in Materials and Methods.

TABLE 4. Effect of temperature on burst size of DD *am* mutants on different *E. coli* strains^a

Phage strain	Nonpermissive hosts (Su ⁻)				Permissive hosts (Su ⁺)			
	<i>E. coli</i> B-S/6		<i>E. coli</i> K12-594		<i>E. coli</i> B-011 ⁺		<i>E. coli</i> K12-CR63	
	25 C	37 C	25 C	37 C	25 C	37 C	25 C	37 C
Gene 39 <i>am</i> E158	0.9 ²	89 ²						
<i>am</i> N116	2.3 ⁴	110 ⁴	59 ²	100 ²	100 ²	163 ²	76 ²	140 ²
<i>am</i> E480	1.2 ²	62 ²	16 ²	66 ²	64 ²	98 ²	56 ²	86 ²
Gene 52 <i>am</i> E177	0.2 ²	30 ²						
<i>am</i> E1240	0.2 ²	47 ²						
<i>am</i> E397	0.9 ²	47 ²	5 ²	51 ²	49 ²	80 ²	49 ²	69 ²
<i>am</i> E670	0.4 ²	39 ²	27 ²	59 ²	66 ²	141 ²	69 ²	98 ²
Gene 58-61 <i>am</i> E822	3.0 ²	58 ²						
<i>am</i> HL627	1.9 ⁴	50 ⁴	29 ²	61 ²	59 ²	110 ²	66 ²	82 ²
<i>am</i> E219	0.8 ²	66 ²	46 ²	89 ²	69 ²	136 ²	86 ²	123 ²
Gene 60 <i>am</i> E429	0.3 ⁴	59 ⁴	49 ²	60 ²	70 ²	98 ²	67 ²	93 ²
<i>am</i> E673	0.1 ²	60 ²						
<i>am</i> HL626	0.9 ²	80 ²	70 ²	101 ²	88 ²	166 ²	95 ²	122 ²
Average	1.0	61	42	75	71	124	71	102
Gene 32 <i>am</i> NG461	0.3 ²	0.5 ²						
T4D ⁺	130 ⁴	150 ⁴	140 ²	176 ²	165 ²	177 ²	160 ²	166 ²

^a Experiments were carried out using the different hosts at 25 and 37 C under otherwise similar conditions. Other details of the experimental procedure appear in Materials and Methods. The values presented are the averages of the number of experiments shown in superscripts.

The Su⁺ strains of *E. coli* permitted much better multiplication of DD *am* mutants than the Su⁻ strains both at 25 and 37 C. The two Su⁺ strains used, *E. coli* 011⁺ and *E. coli* CR63, yielded average burst sizes of 71 each at 25 C, whereas at 37 C the burst size of the mutants was 124 and 102, respectively (Table 4, columns 5, 6, 7, and 8). However, the burst sizes of the mutants on Su⁺ strains were less than the burst size of wild-type T4D⁺. The results in Table 4 taken together indicate that the DD *am* mutants grow substantially better in the presence of an *am* suppressor than in its absence, and at 37 C compared to 25 C. In addition other host-specific factors also appear to enhance phage growth, as indicated by the greater burst size on *E. coli* K (Su⁻) at 25 C compared to *E. coli* B (Su⁻) at 25 C.

Interallelic complementation. Eighteen *am* mutants in gene 39 were tested in 60 pairwise combinations for intragenic complementation. No significant positive responses were observed in any of the tests.

Temperature shift experiments with the DD *am* mutants. As discussed above when the nonpermissive host *E. coli* S/6 is infected with DD *am* mutants at 25 C, very low burst sizes were observed, whereas carrying out the infection at 37 C resulted in much higher burst sizes. To achieve a better understanding of the effect of temperature on burst size, the following temperature shift experiments were performed. *E. coli* S/6 was grown at 25 C prior to infection by

the DD *am* mutants and then shifted to 37 C for infection and subsequently maintained at the higher temperature. Wild-type phage and a cold-sensitive mutant (*csF1* gene 44) were also tested as controls. It was observed (Table 5) that although the temperature during phage growth was high enough for the cold-sensitive gene 44 mutant to give a substantial burst size, the yields of the DD *am* phages were drastically inhibited and no different from that obtained in infections carried out entirely at 25 C (Table 4). The growth of the wild-type phage was not affected by the 25 to 37 C temperature shift. A temperature shift-down experiment was also performed. Cells grown at 37 C prior to infection were shifted to 25 C at the time of infection. The results show that low burst sizes were obtained in this case also (Table 5). The cold-sensitive gene 44 mutant did not have a detectable burst under these conditions and, again, wild-type phage had a near normal burst size.

DNA synthesis by the DD *am* mutants under restrictive conditions. As discussed earlier, Epstein et al. (11) described the DNA defect of the DD mutants as being characterized by a delay in the onset of phage DNA synthesis. DNA synthesis in their experiments was determined by semiquantitative colorimetric measurements. Yegian et al. (31) used radioisotope-labeling and heavy isotope incorporation and found that the apparent delay was due to a lower initial rate of DNA synthesis. However, all of these experiments were carried out at

TABLE 5. *Effect of the temperature shift of the host on the burst size of the DD am mutant phage^a*

Phage strain	Burst size 25 to 37 C shift (1)	Burst size 37 to 25 C shift (2)
Gene 39 <i>am</i> E158	2.2	0.3
Gene 39 <i>am</i> E556	1.9	0.2
Gene 52 <i>am</i> E177	0.1	0.1
Gene 52 <i>am</i> E1240	0.9	0.8
Gene 58-61 <i>am</i> HL627	1.1	1.0
Gene 58-61 <i>am</i> E822	2.6	2.3
Gene 60 <i>am</i> E429	1.2	0.9
Gene 60 <i>am</i> E673	0.1	0.1
Average	1.3	0.7
Gene 44 <i>cs</i> F1	25	0
T4D ⁺	105	96

^a *E. coli* S/6 was grown at 25 C (1) or at 37 C (2) and then shifted to the opposite temperature at the time of infection by the phage. The results given are the averages of two separate experiments in each case. Other details of the experimental procedure appear in Materials and Methods.

relatively high temperatures which according to our observations are partially permissive. Therefore, measurements of DNA synthesis were performed by using radioisotope-labeling in cultures infected at 25 C.

Incorporation of [*methyl*-³H]thymidine into trichloroacetic acid-insoluble material was measured after infection of *E. coli* S/6 at 25 C by a representative *am* mutant of each of the DD genes along with a mutant from a DNA negative gene (gene 41, *am*N57) and wild-type phage. The results shown in Fig. 2 indicate that the extent of thymidine incorporated into DNA in cells infected with DD mutants at 75 min after infection was 3 to 10% of the amount synthesized by wild type, whereas with the DNA-negative mutant the amount was about 3%. The low level of incorporation obtained under nonpermissive conditions indicates that the DD *am* mutants are as deficient in DNA synthesis as are the *am* mutants in other genes classified as DO or DS (11, 28).

Effect of *am* mutations in genes 38, 52, 58-61, and 60 on recombination of *rII* markers. Eight double mutants containing an *am* mutant combined with an *rII* mutant were constructed. The double mutants were then used in crosses as shown in Table 6. Crosses were carried out under semipermissive conditions for the *am* mutation on *E. coli* S/6 at 37 C. It was found (Table 6) that the recombination frequency of the *rII* markers increased 2.1- to 3.5-fold in the double mutants over the control cross of the two *rII* mutants carrying no DD *am* mutation.

Effect of an *am* mutation in the DD genes on reversion of *rII* markers. Double mutants containing both a DD *am* mutation with an *rII* base substitution mutation or with an *rII* frame shift mutation were used to measure the mutator activity of the DD *am* mutant on reversion of the *rII* mutant. The *rII* mutant, *r*1221, is the result of a transition mutation (5) while *r*ED144 and *r*71 are proflavine revertible frame shift mutations (3). Double mutants were grown at 37 C on *E. coli* B/5 which lacks an *am* suppressor and the progeny were determined by plating about 10⁵ phage on *E. coli* CR63λ, a restrictive host for *rII* phage and about 10² phage were plated on *E. coli* CR63 at 37 C. The reversion index was then calculated as the ratio of phage which could plate on *E. coli* CR63 λ at 37 C to the phage that plated on *E. coli* CR63 at 37 C. The results given in Table 7 show that the reversion index of *r*1221 when combined with a DD *am* mutation is 2.6 to 18.7 times the reversion index of *r*1221 when the DD *am* defect is not present. The reversion index for *rII* frame shift mutations increased from 3.8- to 23.7-fold in the presence of the DD *am* defect. When *am*⁺ revertants of *am*HL627*r*ED144 and *am*HL627*r*71 were selected and cultures of these were grown it was found that the reversion of the *rII* frame shift mutations returned approximately to the control value. The reversion indices shown in Tables 6 and 7 are averages of duplicate determinations which generally showed close agreement with each other. The two results shown in Tables 7 and 8 taken together indicate that DD *am* mutations are mutagenic for base substitution and addition-deletion mutations.

Effects of DD *am* mutations on expression of the *ts* mutation in *am ts* double mutants. The two gene 39 *ts* mutants, *ts*A41 and *ts*G41, produce plaques at 25 C but not at temperatures approaching 42 C. Double mutants were constructed with *ts*G41 and DD *am* mutations representative of genes 39, 52, 58-61 and 60.

The *am ts* double mutants obtained were grown in *E. coli* S/6 at 41 C. Each of the single *am* mutants as well as *ts*G41 and T4D⁺ (wild type) were grown under the same conditions. The results of these experiments are shown in Table 9. The burst sizes obtained with *ts*G41 were 6 and 10 phage per bacterium in two experiments. Most of the *am ts*G41 double mutants gave burst sizes in a comparable range. However, the burst sizes obtained from AM-E416(gene 60)*ts*G41(gene 39) and *am*E670(gene 52)*ts*G41(gene 39) produced burst sizes much higher than that obtained from the *ts* mutant itself. The burst sizes in these two cases were

FIG. 2. Total incorporation of [methyl-³H]thymidine into DNA. Samples were taken at 5, 10, 15, 25, 35, 45, 60, 75, and 100 min after infection of *E. coli* S/6. Infection was carried out with *am* mutants representative of the four DD genes and with gene 41 mutant *amN57* and wild type as controls. The rest of the procedure is given in Materials and Methods. The ordinate shows counts per minute $\times 10^{-3}$ [³H]thymidine incorporated into acid-insoluble material. Symbols: ○, gene 39 *amE158*; ▲, gene 52 *amE670*; ○, gene 58-61 *amHL627*; ▼, gene 60 *amE416*; ■, gene 41 *amN57*; Δ, wild-type, T4D⁺.

TABLE 6. *Effect of DD am mutations on recombination of rII mutants^a*

Crosses	Recombination (%) ^b
<i>amE142rED144</i> × <i>amE142r71</i> (gene 39)	2.94
<i>amH17rED144</i> × <i>amH17r71</i> (gene 52)	2.19
<i>amHL627rED144</i> × <i>amHL627r71</i> (gene 58-61)	2.96
<i>amE416rED144</i> × <i>amE416r71</i> (gene 60)	3.63
<i>rED144</i> × <i>r71</i>	1.05

^a The genes in which the *am* mutants are defective are given in parentheses below the cross.

^b Recombination percentages were calculated as (progeny on *E. coli* CR63λ/progeny on *E. coli* CR63) × 200.

TABLE 7. *Effect of am mutation in the DD genes on reversion of an rII base substitution mutation^a*

Phage strain	Reversion index	Factor of increase in reversion index
<i>amE158r1221</i> (gene 39)	148.0 × 10 ⁶	18.7
<i>amE556r1221</i> (gene 39)	36.9 × 10 ⁶	4.7
<i>amE1111r1221</i> (gene 52)	72.5 × 10 ⁶	9.2
<i>amHL627r1221</i> (gene 58-61)	42.1 × 10 ⁶	5.3
<i>amE416r1221</i> (gene 60)	22.9 × 10 ⁶	2.6
<i>r1221</i>	7.9 × 10 ⁶	

^a Genes defective in the *am* mutants are indicated in parentheses. The reversion indices are the averages of duplicate determinations. The procedures used here have been described by Bernstein (3).

comparable to those obtained from the individual *am* mutants, *amE146* and *amE670*, respectively. It is thus apparent that the temperature-sensitive phenotype of *tsG41* is suppressed by *amE416*(gene 60) and *amE670*(gene 52).

DISCUSSION

Host compensation for DD gene functions. Our initial observation was that all *am* mutants defective in each of the four DD genes do not multiply significantly at 25 C on *E. coli* S/6, but that all of them give substantial yields at 37 C (Table 4). This suggests that the DD gene products may be needed only at 25 C, and that a host function can compensate for the defective DD gene products at 37 C. If it is a host function which accounts for growth at 37 C, then the

preinfection growth temperature of the host could be expected to influence the burst size of cells infected with DD *am* mutants. Temperature shift experiments represented in Table 5 show that growth of the host at 25 C prior to infection prevented subsequent growth of the DD *am* mutants at 37 C. This may suggest that a host product, which is made in uninfected cells in an active form at 37 C, but not at 25 C, can compensate during infection for lack of DD gene functions. Since cells grown at 37 C and then shifted to 25 C at the time of infection also have a low burst size, it appears that the active host product becomes inactive when a shift is made to 25 C. The difference in burst size on the two *Su⁻* *E. coli* hosts, S/6 and 594 (Table 4), at 25 C indicates that host-specific factors, possibly related to *E. coli* B-*E. coli* K strain differences, can also affect growth of the DD *am* mutants.

Recently, Mosig et al. (18) have also presented evidence suggesting that the loss of DD genes in incomplete phage chromosomes may be compensated for by a host product.

Possible interaction between the host component and the DD gene products. Two temperature-sensitive mutants are known in genes 39, *tsA41*, and *tsG41* (Fig. 1a) which fail to multiply at higher temperatures. The existence of these *ts* mutants presents an apparent contradiction to our conclusion that the missing DD functions can be compensated for by a host function at higher temperatures. To explain the

TABLE 8. *Effect of am mutations in the DD genes on reversion of rII frame shift mutations^a*

Phage strain	Reversion index	Factor of increase in reversion index
<i>amE142rED144</i> (gene 39)	35.1 × 10 ⁻⁸	3.8
<i>amH17rED144</i> (gene 52)	48.8 × 10 ⁻⁸	5.3
<i>amHL627rED144</i> (gene 58-61)	77.4 × 10 ⁻⁸	8.3
<i>amHL627RrED144</i> (<i>am⁺</i> revertant)	4.46 × 10 ⁻⁸	0.5
<i>rED144</i>	9.29 × 10 ⁻⁸	
<i>amE142r71</i> (gene 39)	31.6 × 10 ⁻⁹	5.3
<i>amH17r71</i> (gene 52)	27.1 × 10 ⁻⁹	4.5
<i>amHL627r71</i> (gene 58-61)	142.0 × 10 ⁻⁹	23.7
<i>amHL627r71</i> (<i>am⁺</i> revertant)	10.3 × 10 ⁻⁹	1.7
<i>r71</i>	5.98 × 10 ⁻⁹	

^a Genes defective in the *am* mutants are indicated in parentheses. The reversion indices are the averages of duplicate determinations. The procedures used here have been described by Bernstein (3).

TABLE 9. Effect of DD *am* mutations on expression of *tsG41* (gene 39) at 41 C

Double mutant	Burst sizes ^a	Single <i>am</i> mutant	Burst sizes ^a	Map position ^b
Gene 39 <i>amE672tsG41</i>	18, 15	<i>amE672</i>	45, 40	0.5
<i>amE205tsG41</i> ^c	12, 10	<i>amE205</i>	75, 65	0.6
<i>amE617tsG41</i>	8, 12	<i>amE617</i>	50, 58	1.7
<i>amE480tsG41</i> ^c	20, 15	<i>amE480</i>	90, 78	9.5
<i>amE556tsG41</i> ^c	9, 6	<i>amE556</i>	105, 85	11.9
<i>amE566tsG41</i> ^c	12, 10	<i>amE566</i>	92, 69	15.7
<i>amE839tsG41</i>	2, 4	<i>amE839</i>	45, 57	15.9
Gene 52 <i>amE670tsG41</i> ^c	80, 89	<i>amE670</i>	89, 75	0
<i>amE663tsG41</i> ^c	8, 6	<i>amE663</i>	95, 75	13.6
<i>amE1111tsG41</i>	4, 4	<i>amE1111</i>	112, 99	14.9
Gene 58-61 <i>amE219tsG41</i> ^c	7, 8	<i>amE219</i>	70, 150	— ^d
<i>amHL627tsG41</i>	18, 12	<i>amHL627</i>	90, 78	
Gene 60 <i>amE416tsG41</i> ^c	121, 98, 80, 90	<i>amE416</i>	80, 70, 69, 75	0
<i>amE673tsG41</i> ^c	9, 4	<i>amE673</i>	60, 73	2.2
<i>amHL626tsG41</i>	15, 8	<i>amHL626</i>	118, 99	3.3
<i>tsG41</i> (gene 39)	6, 10			5.4
T4D ⁺ (wild type)	180, 199			

^a The numbers represent yields in different experiments.

^b Map positions of the *am* mutations within their respective genes are from Fig. 1 and represent the sum of the recombination frequencies between neighboring mutant sites on the respective maps.

^c To verify the presence of the *ts* mutation, *am*⁺ revertants of these double mutants were selected on *E. coli* S/6 at 25 C, and shown to be temperature sensitive.

^d The mutations in gene 58-61 were too few in number and too distant from mutations in other known genes to determine meaningful map positions.

lack of growth at higher temperatures in the case of the two *ts* mutants we postulate that the missense mutations produce a defective protein which interacts with and inactivates the host function.

The efficiency of plating of different DD *am* mutants on *E. coli* S/6 at 37 C varied over a wide range (Table 1) but was found to be reproducible for each mutant and did not depend significantly on undefined factors in the genetic background (Table 3). The differences in efficiency of plating could be interpreted as reflecting differences in interactions of polypeptide fragments produced by the DD *am* mutants with the host product. This interpretation suggests that the wild-type products of the DD genes may normally interact with the host product.

Examples of host-phage product interactions, similar to the one postulated here for host component and DD gene products, have been proposed for both phage λ and phage T4. Georgopoulos and Herskowitz (1971) presented evidence that the product of *E. coli* gene *dnaB* interacts with the P gene product of phage λ to allow normal phage DNA replication. In T4 head morphogenesis, Georgopoulos et al. (12) and Takano and Kakefuda (26) obtained evidence that a membrane-associated host factor interacts with the product of phage gene 31 which is required for phage capsid assembly.

Possible interaction of gene 39 product with the products of genes 52 and 60. In the previous discussion, we presented an interpretation of our data which assumes interaction of the products of the DD genes with a host product. This assumption further suggests that the products of the DD genes may interact with each other, as well as with the host product. To test this hypothesis, *am ts* double mutants were constructed combining *tsG41* (gene 39) with various *am* mutations in genes 52, 58-61, and 60. It was reasoned that if the gene 39 product interacts with the product of the other DD genes as well as with the host product, normal interaction might depend on the presence of all the products. Thus, an *am* mutation in gene 52, 58-61, or 60 might prevent the gene 39 product from interacting with the host product. Since it was assumed that the *ts* phenotype of *tsG41* depends on interaction of the gene 39 product with the host product, it would be expected that *am* mutations in other DD genes, forming only polypeptide fragments, might prevent this interaction, thereby suppressing the *ts* phenotype of *tsG41*. Suppression of the *ts* phenotype would lead to restoration of growth at 37 C on *E. coli* S-6 because the compensating host product would no longer be inactivated.

AmE416, located at the beginning of gene 60 (Fig. 1d), and *amE670*, located near one end of gene 52 (Fig. 1b), were each able to suppress the

ts phenotype of *tsG41* of gene 39. Each of the double mutants, *tsG41amE670* and *tsG41amE416*, gave high burst sizes at 41 C comparable to the burst sizes found in the single *am* infections (Table 9). In other double mutant combinations the burst sizes were low and were comparable to the burst sizes obtained upon infection with *tsG41* alone. As suggested by their map positions, *amE416* and *amE670* may produce short polypeptide fragments corresponding to the amino terminal sequences of their respective genes when infecting an *Su*⁻ host. The two other *am* mutations tested in gene 52 and the two others tested in gene 60 may lead to longer fragments. Jackson and Yanofsky (15) have shown that polypeptide fragments formed by *am* mutants can interact with the complementary homologous polypeptides produced by *ts* mutants. If the wild-type products of two or more nonhomologous genes ordinarily interact, it may also be expected that polypeptide fragments produced by *am* mutations in one of the genes will interact with the wild-type products of the other gene(s) provided the fragments are long enough. Short fragments might not have the specificity to interact with the other protein(s). Thus, in the cases of *amE670* and *amE416*, it is reasoned that very short polypeptides were synthesized, and consequently a required interaction could not occur. When *amE670* or *amE416* were combined with the *tsG41* mutation, the temperature-sensitive gene 39 protein could not interact with the host product and inactivate it. It then follows that the other *am* fragments, which were probably longer, did allow the *tsG41* protein to inactivate the host product.

Defects in the DD genes affect replication, recombination, and mutation. Epstein et al. (11) originally described the defect of the DD mutants as a delay in DNA synthesis. This description was recently modified by Yegian et al. (31) who showed that a lowered initial rate of DNA synthesis caused the apparent delay. These two groups of workers carried out their experiments at a temperature of 37 C where the host provides compensation. At the more restrictive temperature of 25 C on the nonpermissive host *E. coli* S/6, we observed that the DD mutant infections result in only 3 to 10% of the level of wild-type T4 phage DNA synthesis at late times (Fig. 2). By comparison, under the same conditions, *amN57* (gene 41) classified as a DNA-negative mutant (11) gave 3% of normal DNA synthesis. Thus, under restrictive conditions, the DD *am* mutations appear to be associated with processes essential to DNA synthesis.

Berger et al. (2) showed that a gene 58-61 *am* mutant increased recombination about three- to fourfold. Our results (Table 6) show that *am* mutations in all of the four DD genes cause increased recombination. In addition, we have observed that DD *am* mutations are mutagenic, causing both increased base substitution mutation (Table 7) and increased addition-deletion mutation (Table 8). These increases in recombination and mutation suggest that the host compensated DNA synthesis is less accurate than that directed by wild-type phage. Yegian et al. (31) found that the DNA synthesized on *E. coli* S/6 at 37 C was about 70% of the wild-type levels. Naot and Shalitin (19) investigated the intracellular DNA formed following infection of a nonpermissive host by wild-type T4 phage and by *am* mutants defective in genes 39, 52, 58-61, and 60. In this case the infections were at 25 C for 2 min followed by transfer to 37 C. They extracted DNA from infected cells and analyzed the DNA in alkaline sucrose gradients. It was observed that the single-stranded DNA from the DD *am* mutant infections was shorter than the mature phage chromosome length, whereas the single strands of intracellular DNA from wild-type infections were several fold longer than the mature chromosome. These results further indicate that DNA replication directed by host product after infection by DD *am* mutants is abnormal.

Possible interaction of the DD gene products with the host membrane. The DD genes 39, 52, and 60 are located in that region of the T4 genetic map which contains several other genes which have been shown either to modify the host membrane, such as *rIIA* (10) and *rIIB* (22, 29), or to have membrane related functions such as the *ac* gene (8, 24) and gene *t* (16).

Guttman and Begley (13) have obtained evidence suggesting that an *am* mutation in gene 39 causes impaired uptake of Mg^{2+} ions in infected cells. Dion and Cohen (7), using the same *am* mutation, obtained similar results suggesting that spermidine uptake is also impaired. Naot and Shalitin (20) and E. Earhart (personal communication) have obtained evidence that on infection by DD *am* mutants the intracellular phage DNA bound to the membrane is prematurely released from its membrane association.

The chemical and functional properties of the membrane have been shown to vary with temperature of growth (14). A marked increase in saturated, and a decrease in unsaturated fatty acids was observed when the temperature of growth was elevated. The increased compensation for lacking DD gene functions with increas-

ing temperature might be related to this temperature-dependent property of the membrane.

From our results and the evidence cited in this section, we suggest that the products of the DD genes and a host component interact at the membrane.

Comments on previous interpretations of the DD am mutant phenotype. Previous work in this laboratory reported by Vallée and Cornett (27) included pairwise complementation spot tests of the gene 39 *am* mutations performed at 25 C. Some of these tests were positive, a result not expected if the mutants are true *amber* nonsense mutations. To check these results, 18 gene 39 *am* mutants were tested for intragenic complementation in 60 mixed infections and the burst sizes were measured. None of these tests showed significant positive complementation. Since burst size is a much more reliable measure of complementation than the spot test method, it is concluded that the gene 39 *am* mutations behave as true nonsense mutations.

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