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New Late Gene, *dar*, Involved in DNA Replication of Bacteriophage T4

I. Isolation, Characterization, and Genetic Location

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Suppressors of gene 59-defective mutants were isolated by screening spontaneous, temperature-sensitive (*ts*) revertants of the *amber* mutant, *am*C5, in gene 59. Six *ts* revertants were isolated. No gene 59-defective *ts* recombinant was obtained by crossing each *ts* revertant with the wild type, T4D. However, suppressors of gene 59-defective mutants were obtained from two of these *ts* revertants. These suppressor mutants are referred to as *dar* (DNA arrested restoration). *dar* mutants specifically restored the abnormalities, both in DNA synthesis and burst size, caused by gene 59-defective mutants to normal levels. It is unlikely that *dar* mutants are nonsense suppressors since they failed to suppress *amber* mutations in 11 other genes investigated. The genetic expression of *dar* is controlled by gene 55; therefore, *dar* is a late gene. The genetic location of *dar* has been mapped between genes 24 and 25, a region contiguous to late genes. *dar* appears to be another nonessential gene of T4 since burst sizes of *dar* were almost identical to those of the wild type. Mutations in *dar* did not affect genetic recombination and repair of UV-damaged DNA, but caused a sensitivity to hydroxyurea in progeny formation. The effect of the *dar* mutation on host DNA degradation cannot account for its hydroxyurea sensitivity. *dar* mutant alleles were recessive to the wild-type allele as judged by restoration of arrested DNA synthesis. The possible mechanisms for the suppression of defects in gene 59 are discussed.

Synthesis of DNA requires nucleoprotein complexes that contain cell membrane, nucleic acids, and various structural and catalytic proteins. The synthetic process can be divided into at least three steps: (i) initiation, (ii) elongation, and (iii) termination. Each step is complicated and appears to have its own specific control mechanisms in addition to the overall regulatory mechanisms.

After the initiation of DNA synthesis, the nature of DNA structure (circular or linear, etc.), the integrity of genetic recombination and replicative (a term designed for the repairing process observed in normal DNA replication) repair, and the stability of the replicative complex affect the further development of DNA replication. The organization and regulation of these factors in vivo are still unknown.

We have shown that *amber* mutants defective in gene 59 (DA type) of phage T4 cause an arrest of DNA synthesis 2 to 3 min after initiation, a premature release of DNA from the replicative complex, and a decrease in the rate of replicative repair and prevent concatemer formation in a nonpermissive host (27, 29). However, an extragenic mutation in either gene 55 or 33 (MD type) or the addition of chloramphenicol 5 to 12 min after infection results in the restoration of DNA synthesis to the normal level. These results indicate that at least one of the late genes is involved in this maintenance of the DNA arrest phenotype at late times. However, mechanisms for the biological functions of this late gene(s) under normal conditions are unknown.

This paper reports the isolation, genetic mapping, and partial characterization of such a late gene that can overcome or suppress the DA phenotype of mutants defective in gene 59. There has been a preliminary account of some of these experiments (Y. C. Yeh and J. R. Wu, Cold Spring Harbor Phage Meet., p. 25, 1973).

MATERIALS AND METHODS

Media. 1 × C medium (28) was used in [^{14}C]- or [^{3}H]thymine incorporation experiments. 3 × D medium was used in preparing bacteriophage lysate. This medium was prepared by mixing sterilized

solution A (containing 9 g of KH_2PO_4, 21 g of Na_2HPO_4, 6 g of NH_4Cl, 30 g of casein hydrolysate, 26 g of glycerol, and 60 mg of gelatin in 1,900 ml of distilled water) and sterilized solution B (containing 0.6 g of $MgSO_4 \cdot 7H_2O$ and 0.6 ml of 1 M $CaCl_2$ in 100 ml of water). Nutrient broth medium, which was made by mixing 8 g of nutrient broth and 5 g of NaCl in 1 liter of distilled water, was used for genetic crosses, determination of burst size, and growing the *Escherichia coli* culture.

Bacteria and bacteriophage. *E. coli* K strain CR63, which carries su^+, was used as permissive host for T4 *amber* (*am*) mutants and for genetic crosses. *E. coli* B strain Tr201, a low-thymine-requiring strain obtained from G. R. Greenberg, was used as a nonpermissive host for T4 *amber* mutants in [^{14}C]- or [^{3}H]thymine incorporation experiments. *E. coli* B021 was used as a nonpermissive host in determination of burst size. *E. coli* BB was used as a nonpermissive host in isolation of mutants, spot complementation test, and genetic mapping.

Bacteriophage T4D was used as wild type in all experiments. T4 *amber* mutants used, all of which were derived from T4D, are listed in Table 1. Two gene 59-defective *amber* mutants (*am*C5 and *am*HL628) were obtained from R. S. Edgar. In most cases, *am*C5 was used. This mutant was purified by backcross with the wild type, T4D. Late mutants were provided by W. B. Wood. R9 was obtained from J. Karam, and *y* and *v* mutants were obtained from K. Ebisuzaki.

Genetic mapping. The procedure reported by Tessman (22) was used.

Spot complementation test. The procedure described by Edgar was used (5).

Measurement of the kinetics of DNA synthesis. The procedure has been described previously (27).

Construction of multiple phage mutants. Double *amber* mutants were constructed by standard procedures. In constructing triple mutants, *dar-am-am*C5 (*am* stands for any *amber* mutant constructed), the procedure described by Hercules and Wiberg was used (10). When the map location of *am* was closed to *dar*, the *dar-am-am*C5 triple mutant was distinguished from the *am-am*C5 double mutant by measuring the kinetics of DNA synthesis. If the *am* was not a mutant of DO or DA type, the triple mutant would continually synthesize DNA whereas the double mutant would be arrested in DNA synthesis.

To construct *dar-am* double mutants, *dar-am-am*C5 was crossed with *dar*. *amber* mutants including only *dar-am*C5 and *dar-am-am*C5 were selected from lysate. *dar-am* and *dar-am-am*C5 were distinguished by the kinetics of DNA synthesis. Since the *dar* mutant allele was recessive in function, DNA synthesis was arrested in nonpermissive host cells infected with a phage mixture containing equal ratios of *dar-am-am*C5 and *am*C5. In contrast, DNA was continuously synthesized in cells infected by the mixture containing equal ratios of *dar-am* and *am*C5.

Analysis of alkaline sucrose density gradients. The treatment of infected cells, the lysing procedure, and conditions for sedimentation have been reported previously (28). The lysing procedure described by Miller et al. (17) was used.

UV irradiation. Two milliliters of the infected culture was placed on a petri dish (5.25-cm diameter) with shaking and was irradiated with a 15-W germicidal lamp (General Electric) at room temperature. The UV dosage (700 ergs/mm^2) was measured by Blak-Ray UV meter (model J-225; UV Products, Inc.).

Spot test for hydroxyurea (HU) sensitivity. The procedure reported by Karam and Bowles (12) was performed.

RESULTS

Isolation of temperature-sensitive mutants from pseudo-revertants of amC5 (gene 59). A screening procedure based on spontaneous reversion was performed to isolate temperature-sensitive (*ts*) mutants or extragenic suppressors of gene 59. Among the spontaneous revertants of *am*C5, two possible genotypes should be included. One is the revertant in which the nonsense codon is reverted to wild type or pseudo-wild type. Some of these pseudo-wild types may be *ts* mutants if the nonsense mutation of *am*C5 is located in a region coding for information that is essential for maintaining the conformation of gene 59 product. The other genotype is the revertant in which the nonsense codon is not changed but another extragenic point mutation is spontaneously formed, and this extragenic mutation causes the restoration of arrested DNA synthesis. Therefore, the analysis of spontaneous revertants of *am*C5 theoretically provides not only a simple way to select *ts* mutants of gene 59 but also an approach to isolate the suppressors (3; I. Tessman, personal communication).

TABLE 1. *T4 amber mutants used*

Gene	Mutant	Mutant phenotype or known product
46	*am*N130	DNA arrest, exonuclease
47	*am*A456	DNA arrest, exonuclease
55	*am*BL292	Maturation defective, control late gene function
49	*am*E727	Nucleocapsid maturation blocked, endonuclease
22	*am*B270	Minor head component
23	*am*B17	Major head component
	*am*B272	
24	*am*N65	Minor head component
25	*am*S52	Baseplate formation blocked
26	*am*N131	Baseplate formation blocked
30	*am*HX39	Polynucleotide ligase
59	*am*C5	DNA synthesis arrested
	*am*HL628	
45	*am*E10	No DNA synthesis

A stock of *am*C5 was plated on a nonpermissive host, *E. coli* BB, at room temperature. The spontaneous revertants with large plaques were picked up and their temperature sensitivity was tested on *E. coli* BB at 41 and 25 C. Six out of 20,000 revertants tested were temperature sensitive and were referred to as *ts*591 through *ts*596.

The nature of reversion in these *ts* revertants was analyzed by examining recombinants obtained after crossing each *ts* revertant with the wild type, T4D. We have found that all the original *ts* revertants contained an *amber* mutation in gene 59 as evidenced by the complementation test and kinetics of DNA synthesis in a nonpermissive host.

Isolation of the suppressor of amC5 from ts revertants. Among recombinants from the cross of *ts*596 with the wild type, T4D, we found a recombinant that contained *am*C5 but grew well in *E. coli* BB at both high and low temperature (41 and 25 C). This phenotype probably resulted from the crossing out of the unknown *ts* mutational site and the creation of a recombinant containing *am*C5 and its extragenic suppressor. This recombinant, referred to as R6, was backcrossed twice with *am*C5 to cross out any other silent mutation. For further analysis, R6 was crossed with T4D and the recombinants were analyzed. *am*C5 and a new type of recombinant that does not contain an *amber* nonsense mutation were found among the progeny recombinants. This new type of phage formed wild-type plaques on *E. coli* BB but smaller plaques on *E. coli* CR63. This mutant was designated as *dar*1 (DNA arrested restoration). A double mutant containing *dar*1 and *am*C5 was reconstructed by crossing *dar*1 with *am*C5. The existence of *am*C5 in this reconstructed double mutant was confirmed by backcrossing this double mutant with T4D again. *am*C5 was found in high frequency among the progeny. The double mutant, *dar*1-*am*C5, is identical to R6 as evidenced by their growth and kinetics of DNA synthesis in *E. coli* B strain. The kinetics of DNA synthesis of *dar*1-*am*C5, R6, and *dar*1 in *E. coli* B Tr201 is shown in Fig. 1. In contrast to *am*C5, their kinetics of DNA synthesis was similar to that of T4D, i.e., continuous synthesis. Since *dar*1 grew better in *E. coli* B than in *E. coli* CR63, it is obvious that *dar*1 is a nonlethal missense mutant.

The other nonsense gene 59-defective mutant, *am*HL628, was also tested with *dar*1. The double mutant, *dar*1-*am*HL628, had the same phenotype shown by *dar*1-*am*C5.

The suppression of gene 59-defective mutants by *dar*1 described above is based on DNA synthesis and growth in *E. coli* B strains (nonpermissive host for *amber* mutants). More convincing evidence would be the results obtained from the analysis of burst size (Table 2). The burst size of gene 59-defective mutants (*am*C5 or *am*HL628) was about 20 in a nonpermissive host. The addition of *dar*1 to gene 59-defective mutants increased burst size to about 200, 80% of that observed in wild type. *dar*1 itself also

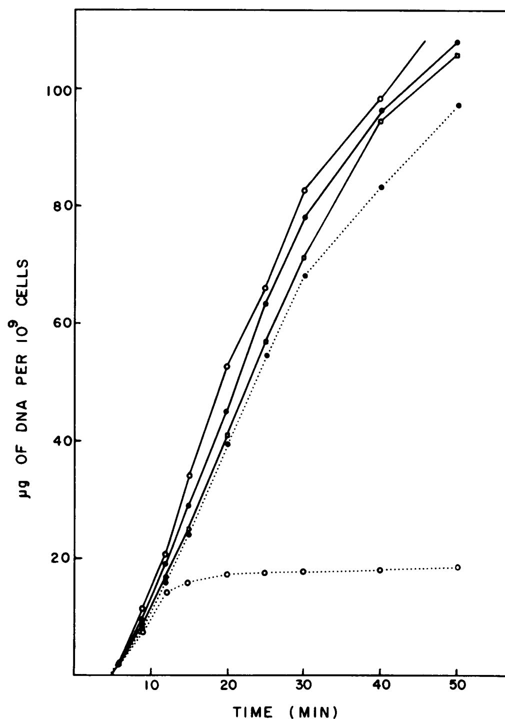

FIG. 1. *Kinetics of DNA synthesis in E. coli B Tr201 cells infected by dar1, R6, or dar1-amC5 at 37 C. MOI = 5. Symbols: --●--, T4D; --○--, amC5; —□—, dar1; —○—, R6; —●—, dar1-amC5.*

TABLE 2. *Suppression of gene 59 mutants by dar1 as measured by burst size*

Phage	Burst size[a]	
	B021	CR63
T4D	258	230
*am*C5	25	211
*am*HL628	21	187
*dar*1-*am*C5	213	183
*dar*1-*am*HL628	187	173
*dar*1	198	171

[a] Phage produced per infected cell at 37 C in 50 min.

had a burst size about 200. This result shows that gene 59-defective mutations were suppressed by *dar1* and that a mutation in *dar1* did not cause a lethal effect on growth. Therefore, it appears that *dar* is a nonessential gene.

By the same procedure, *dar2* was isolated from *ts595*. The phenotypes of *dar2* were similar to those of *dar1*. There was evidence that *dar1* and *dar2* are located in the same cistron (see below).

Genetic location of dar. It is difficult to distinguish *dar* from wild type by the plaque morphology. Therefore, a procedure that permits the growth of only one of the recombinants but not the other or parental phages was applied. This procedure has been reported by Hercules and Wiberg (10) to map their *das* mutants. A triple mutant, *dar-am-amC5*, was crossed with *amC5* in a permissive host, *E. coli* CR63 (*am* in the triple mutant indicates any *amber* mutant constructed). In this cross, the parental type progeny, *dar-am-amC5* and *amC5*, and the recombinant, *dar-am*, made no plaque or tiny plaques (in the case of *amC5*) on the nonpermissive host *E. coli* BB, but the recombinant, *dar-amC5*, made plaques as large as those of the wild type. Therefore, recombination frequency between *dar* and *am* can be determined by this procedure. The results of these two-factor crosses indicate that *dar1* and *dar2* are located between genes 24 and 25 (Table 3). *dar1* was mapped 9.6 units counterclockwise to *amS52* (gene 25) and 14.6 units clockwise to *amN65* (gene 24). *dar2* was mapped very close the to *dar1* (less than one map unit). This suggests that *dar1* and *dar2* are probably located in the same cistron. This assumption was confirmed by the result of the complementation test, which is based on wild-type allele dominance of physiological function. *E. coli* B Tr201 was infected by a phage mixture containing equal ratios of *dar1-amC5* and *dar2-amC5* (multiplicity of infection [MOI] at 5 each). If *dar1* and *dar2* are in the same cistron, DNA should be continuously synthesized in the infected cells. On the other hand, if *dar1* and *dar2* are in different cistrons, they should complement each other and allow the formation of active *dar* protein. Therefore, DNA synthesis should be arrested in infected cells. The result of this experiment showed that DNA was continuously synthesized in infected cells, indicating that *dar1* and *dar2* are located in the same cistron. Although *dar1* and *dar2* are in the same cistron, they are not at an identical mutational site since *amC5* recombinant was isolated from the cross of *dar1-amC5* with *dar2-amC5* at low frequency. Because *dar1* and *dar2* are located in the same cistron, *dar1* was used in most later experiments.

To determine the exact map order of *dar* among genes 24, 25, and 26, three-factor crosses were performed by crossing *dar1-am*[a]*-amC5* with *am*[b]*-amC5* (a and b indicate different mutations used) and the recombinant, *dar1-amC5*, was scored for determining recombination frequency (Table 4). These results also indicate that *dar* is located between genes 24 and 25, since only in this order can the lowest

TABLE 3. *Two-factor crosses performed to established the map position of dar*

Phage mutants in cross[a]	Recombination (%)	Interval determined
dar1-amBL292-amC5 × *amC5*	36.2[b]	*dar1*-55
dar1-amB17-amC5 × *amC5*	16.5 ± 0.4[c] (4)[d]	*dar1*-23
dar1-amN65-amC5 × *amC5*	14.6 ± 0.4 (4)	*dar1*-24
dar1-amS52-amC5 × *amC5*	9.6 ± 0.2 (4)	*dar1*-25
dar1-amN131-amC5 × *amC5*	11.8 ± 0.6 (5)	*dar1*-26
dar1-amHX39-amC5 × *amC5*	24.8[b]	*dar1*-30
dar2-amN65-amC5 × *amC5*	13.0 ± 1.4 (4)	*dar2*-24
dar2-amS52-amC5 × *amC5*	10.8 ± 0.3 (4)	*dar2*-25
dar1-amB17-amC5 × *dar1-amN65-amC5*	9.5 ± 0.9 (4)	23-24
dar1-amN65-amC5 × *dar1-amS52-amC5*	20.3 ± 0.6 (3)	24-25
dar1-amS52-amC5 × *dar1-amN131-amC5*	4.2 ± 0.5 (4)	25-26
amB17 × *amN65*	9.0[b]	23-24
amB17 × *amN65*[e]	9.8[b]	23-24
amB17-amC5 × *amN65-amC5*	9.6[b]	23-24
dar1-amB17-amC5 × *dar1-amN65-amC5*[e]	9.8[b]	23-24

[a] Crosses were carried out in *E. coli* CR63 except those indicated by superscript *e*, which were in *E. coli* B021.

[b] Mean of two crosses.

[c] Standard error of mean.

[d] Number of crosses.

[e] The cross was carried out in *E. coli* B021.

TABLE 4. *Three-factor crosses*

Mutants in cross	Recombination frequency[a]
dar1-amS52-amC5 × *amN65-amC5*	7.4
dar1-amN65-amC5 × *amS52-amC5*	10.7
dar1-amN131-amC5 × *amS52-amC5*	2.6
dar1-amS52-amC5 × *amN131-amC5*	0.8

[a] The recombinant, *dar1-amC5*, was scored for determining recombination frequency. The figures presented are the mean values of two experiments.

recombination frequency that results from a double cross be found in the cross of *dar*1-*am*S52 (gene 25)-*am*C5 with *am*N131 (gene 26)-*am*C5.

dar did not affect DNA recombination, as shown by recombination frequencies observed between genes 23 and 24 in various crosses (Table 3). *E. coli* CR63 suppressed only the *amber* nonsense mutation but not the missense mutation, which includes *dar*. Therefore, active *dar* protein was not present in *E. coli* CR63 cells infected by the *dar*1 mutant. By using *E. coli* CR63 as host, we observed recombination frequencies between genes 23 and 24 of about 9.5 in the cross of *dar*1-*am*B17 (gene 23)-*am*C5 with *dar*1-*am*N65 (gene 24)-*am*C5; 9.6 in the cross of *am*B17-*am*C5 with *am*N65-*am*C5; and 9.0 in the cross of *am*B17 with *am*N65. These results suggest that the recombination frequencies between genes 23 and 24 are not significantly affected by the presence or absence of active *dar* protein in the infected cells. The genetic location of *dar* relative to other genes on T4 chromosome is shown in Fig. 2.

Specificity of dar in the suppression of gene 59-defective mutants. It has been reported that eight tRNA's are coded by the T4 genome (4, 19). It is possible that *dar* is mutated in a phage-coded tRNA (26), which results in a suppression of all *amber* mutants including *am*C5 and *am*HL68. This possibility is ruled out by the fact that *dar* mutants do not suppress *amber* mutations in other genes so far tested. These include genes 22, 23, 24, 25, 26, 30, 45, 46, 47, 49, and 55. During the process of mapping for *dar*, various triple mutants containing *dar*, *am*C5, and *amber* mutants in all genes described above were constructed. Except for a triple mutant, *dar*1-*am*E727 (gene 49)-*am*C5, which will be described in a separate paper, all other triple mutants showed *amber* characteristics. This indicates that the *dar* mutation is unable to suppress *amber* condons and is not identical to a tRNA mutation, which nonspecifically suppresses *amber* condons. The gene 49-defective mutant is not suppressed by *dar* mutation. A double mutant, *dar*1-*am*E727, was constructed. This double mutant is unable to grow on *E. coli* B strains.

Trans-dominance of the wild-type allele. By infecting a nonpermissive host with a mixture of *dar*1-*am*C5 and *am*C5 and studying the kinetics of DNA synthesis in this infected culture, one can determine whether the wild-type

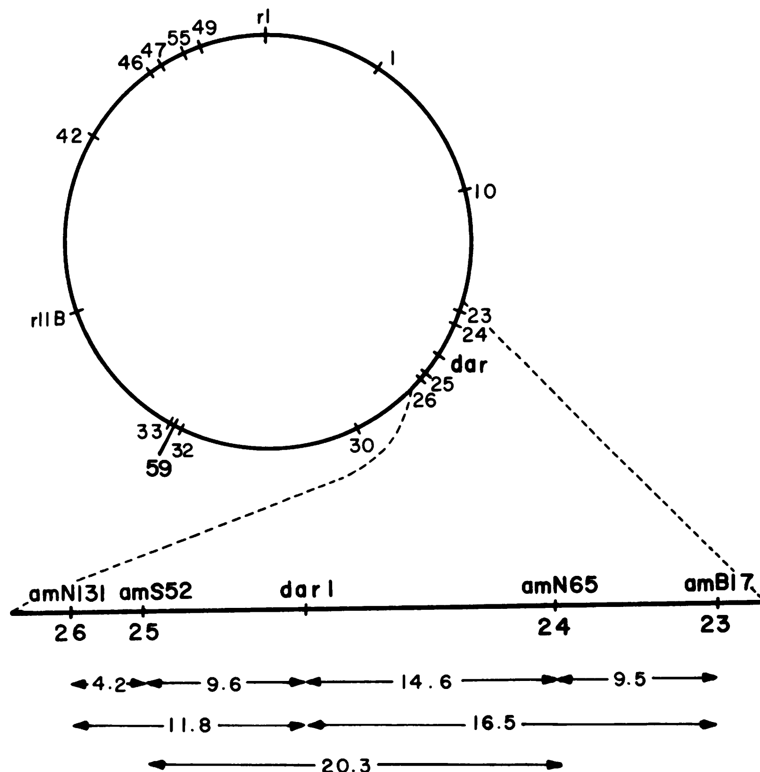

FIG. 2. *Genetic location of dar.*

or mutant allele is functionally dominant. If the *dar* mutant allele is dominant, the kinetics of DNA synthesis in infected cells should be similar to that of *dar*1-*am*C5; i.e., DNA should be continually synthesized. On the other hand, if the wild-type allele is dominant, the kinetics of DNA synthesis should follow that of *am*C5; i.e., DNA synthesis should be arrested. The result of this experiment is shown in Fig. 3. It is evident that the kinetics of DNA synthesis followed that of *am*C5, i.e., an arrest of DNA synthesis. Even though the ratio of MOI of *dar*1-*am*C5 to *am*C5 was as high as 4, this phenomenon still could be seen. This result strongly indicates that the wild-type allele is transdominant over the mutant allele.

dar is a late gene. Pulitzer and Geiduschek (18) have shown that gene 55 protein is continuously required for late transcription. It has been suggested that gene 55 protein might function by binding to host RNA polymerase and then by altering its specificity from early to late mRNA formation (23). Stevens (21) proved this hypothesis by directly isolating this binding factor from RNA polymerase isolated after T4 infection. Therefore, if *dar* is a late gene, its expression should be controlled by gene 55. To test whether or not gene 55 controls the expression of *dar*, the following experiment was carried out. *E. coli* B Tr201 was infected by a phage mixture containing two double mutants, *dar*1-*am*C5 and *am*BL292 (gene 55)-*am*C5, at an MOI at 5 each, whereas in the control experiment, cells were infected by a phage mixture containing a triple mutant, *dar*1-*am*BL292-*am*C5, and a double mutant, *am*BL292-*am*C5, at an MOI of 5 each. The kinetics of DNA synthesis in both infected cultures (experimental and control) was measured. If gene 55 controls the expression of *dar*, the kinetics of DNA synthesis in the experimental infection should be of an arrested type. This is because gene 55 is not defective in the double mutant, *dar*1-*am*C5, and the active 55 protein allows gene *dar* in another double mutant, *am*BL292-*am*C5, to be expressed as an active product. Once active *dar* protein is present in infected cells, DNA synthesis should be arrested (based on the trans-dominance of the wild type [Fig. 3]). On the other hand, if gene 55 controls the expression of *dar*, DNA synthesis in the control experiment should continue. In this case, gene 55 is defective in both the triple mutant, *dar*1-*am*BL292-*am*C5, and the double mutant, *am*BL292-*am*C5. Therefore, the expression of late genes will not be turned on. *dar* protein would not appear in infected cells, although gene *dar* is not defective in the double mutant, *am*BL292-*am*C5. This absence of *dar* protein allows DNA to be continually synthesized in infected cells. The result of this experiment is shown in Fig. 4. In the case of the experimental infection, DNA synthesis was arrested. This arrest was not caused by either *dar*1-*am*C5 or *am*BL292-*am*C5. In both cases, when they were infected separately, DNA was continuously synthesized. On the other hand, in the control infection, DNA was continuously synthesized in infected cells. Therefore, it is obvious that the expression of *dar* is controlled by gene 55. These results strongly indicate that *dar* is a late gene.

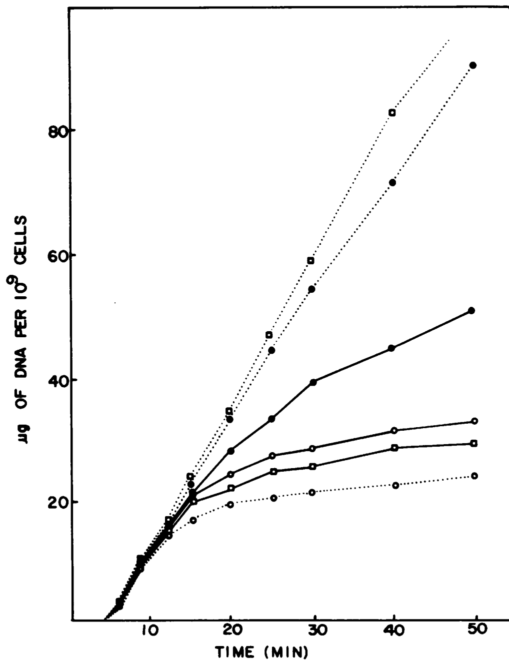

FIG. 3. *Kinetics of DNA synthesis at 37 C for determining the trans-dominance of the dar⁺ allele (see text). A total MOI of 10 was used in each infection. Symbols: --●--, T4D; --○--, amC5; --□--, dar1-amC5; —●—, dar1-amC5:amC5 = 4:1; —○—, dar1-amC5:amC5 = 1:1; —□—, dar1-amC5:amC5 = 1:4.*

Sensitivities to UV irradiation or HU. Two of the other three mutants located between genes 24 and 25 are *y*(20) and *m*22 (N. V. Hamlett and H. Berger, Cold Spring Harbor Phage Meet., p. 24, 1973). Mutant *y* was reported to be UV sensitive and to cause a decrease in genetic recombination (2). In addition to being UV sensitive and causing a decrease in genetic recombination, *m*22 was reported to be HU sensitive. To compare *dar* with *y* or *m*22, UV and HU sensitivities of *dar* were tested.

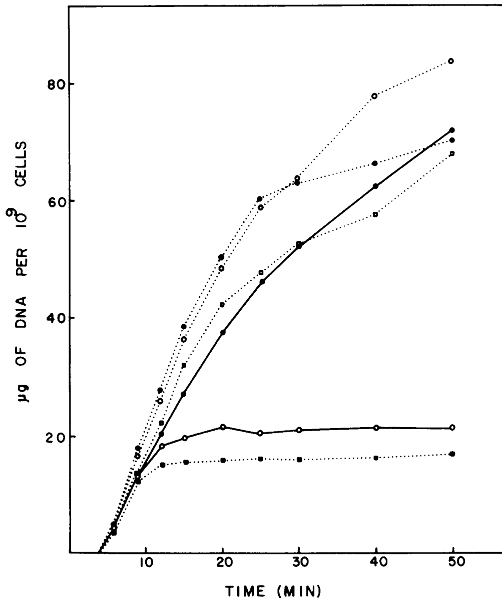

FIG. 4. *Kinetics of DNA synthesis at 37 C for determining the late function of dar (see text). A total MOI of 10 was used in each infection. In the mixed infections, MOI was 5 for each phage mutants. Symbols: —O—, dar1-amC5 + amBL292-amC5; —●—, amBL292-amC5 + dar1-amBL292-amC5; - -O- -, dar1-amC5; - -□- -, amBL292-amC5; - -●- -, dar1-amBL292-amC5; - -■- -, amC5.*

UV sensitivity. The result of the survival-UV dose experiment is shown in Fig. 5. The survival curve of *dar* tested in either *E. coli* BB or *E. coli* CR63 was similar to that of the wild-type control, T4D, whereas two UV-sensitive mutants, *y* and *v*, showed about twice the sensitivity of T4D (relative slop in *E. coli* BO21: T4D = 0.63; *dar*1 = 0.70; *y* = 1.1; *v* = 1.2). This result indicates that *dar* is not sensitive to UV irradiation. This conclusion was further confirmed by the following experiment. The time-dependent change in sedimentation rate of single-stranded DNA in alkaline sucrose density gradients reflects the degree of repair of UV-damaged DNA and can be used as a parameter to determine the UV sensitivity. The infected cells were labeled with [^{14}C]thymine from 4 to 10 min after infection at 33 C, followed by UV irradiation (700 ergs/mm^2) and then chased with a medium containing a high concentration of cold thymine (2 mg/ml). At various intervals after chasing, intracellular DNA was extracted under alkaline conditions (0.4 N NaOH) and sedimented in alkaline sucrose density gradients (Fig. 6). After chasing for 40 min, UV-damaged DNA in the *dar* preparation was repaired and sedimented with standard T4 DNA, though the rate of repair in *dar* was slower than that of the wild type, T4D. This result is further indication that *dar* is not UV sensitive.

HU sensitivity. Spot test for HU sensitivity showed that both *dar*1 and *dar*2 were sensitive to HU. *dar* mutants made nonlysed spots on the bacterial lawn in the presence of HU, whereas the wild type yielded completely lysed spots (clear). An HU-sensitive mutant, R9 (12), was also used as a negative control. However, R9 yielded turbid spots on the bacterial lawn. This result indicates that *dar* mutants are sensitive to HU and are more sensitive than R9.

Burst size in the presence of various concentrations of HU is shown in Table 5. The burst size of the control, T4D, gradually decreased when the concentration of HU was increased and finally decreased to about 20 at higher concentrations, a result consistent with the previous report by Warner and Hobbs (24). In the case of *dar*1, the degree of decrease in burst size with HU concentration was observed to be greater than that of the wild type. At 20 mM HU, almost no progeny was formed (burst size = 0.24). This difference can be seen more clearly in the burst size ratio of *dar*1 to T4D (Table 5, column 4). In the absence of HU, the ratio was 0.75. The ratio gradually decreased as the concentration of HU increased and reached 0.01 at 20 mM HU. This result strongly indicates that *dar*1 is an HU-sensitive mutant.

A defect in degrading the host DNA is found in a number of HU-sensitive mutants (9, 25).

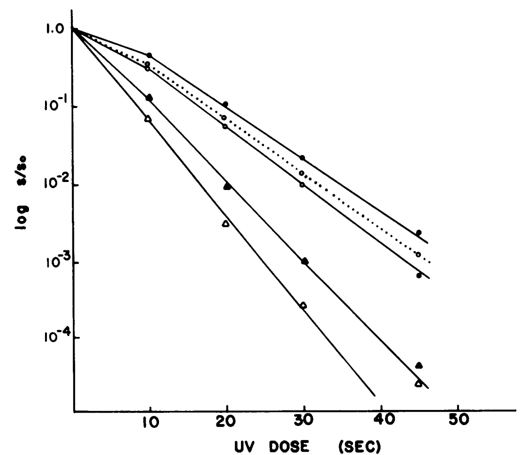

FIG. 5. *UV survival curves of dar1 on E. coli BO21 or E. coli CR63. UV dosage = 12 erg/mm^2 per s. Symbols: —O—, dar1 on E. coli BO21; - -O- -, dar1 on E. coli CR63; —●—, T4D on E. coli BO21; —▲—, y on E. coli BO21; - -△- -, v on E. coli BO21.*

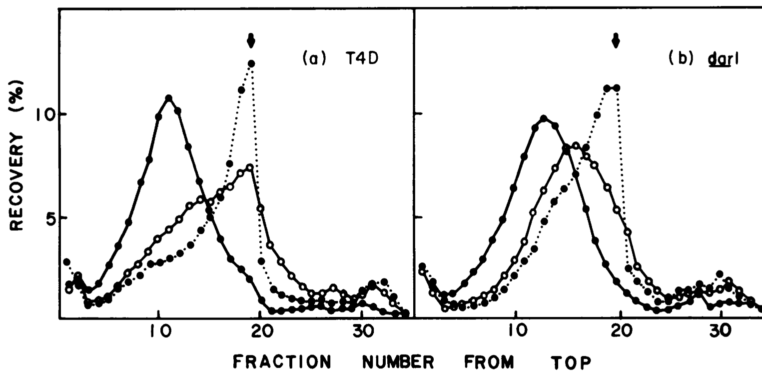

FIG. 6. *Sedimentation profiles of [^{3}H]thymine-labeled DNA by alkaline sucrose gradient centrifugation. Samples were extracted after infected cells were UV irradiated (700 ergs/mm^2) and chased with cold thymine at 10 min in 33 C. (a) T4D DNA; (b) darl DNA. Symbols: —●—, Chased for 1 min; —○—, chased for 20 min; - -●- -, chased for 40 min. Arrows indicate the sedimentation position of single-stranded T4 DNA.*

TABLE 5. *Burst size of darl in various concentrations of hydroxyurea*

Hydroxyurea (mM)	Burst size[a]		Ratio (*dar*1/T4D)
	T4D	*dar*1	
0	160	120	0.75
0.3	85.7	59.3	0.69
0.6		38.9	
1		24.9	
1.5	41.5	20.4	0.49
3	28.6	10.4	0.36
6	24.4	4.7	0.19
10	23.2	2.4	0.10
20	20.0	0.24	0.01

[a] Phage produced per infected cell at 37 C in 60 min in *E. coli* B021.

Therefore, the degradation of host DNA in *dar*1-infected cells was measured. Since degraded products of host DNA might be reutilized by viral DNA synthesis in *dar*1-infected cells, a mutant of DO type (no DNA synthesis), *am*N122 (gene 42, coded for dCMP hydroxymethylase), was introduced into *dar*1. Bacterial DNA was labeled with [^{3}H]thymine for two generations, centrifuged twice to wash out free [^{3}H]thymine before infection, and then infected with phage at an MOI of 8. At various intervals after infection, the radioactivity of the acid-insoluble material was measured. Host DNA was degraded to 40% in the control, *am*N122 (Fig. 7). Mutant *dar*1 only slightly affected the degradation of host DNA, and 50% was reached in *dar*1-*am*N122-infected cells. However, this slight effect on host DNA degradation cannot account for the HU sensitivity of *dar* mutants.

If the DO mutant was not introduced to *dar*1, the radioactivity of acid-insoluble material did not disappear in *dar*1-infected cells with time (Fig. 7). This result indicates that the degraded host DNA in *dar*1-infected cells is efficiently reutilized to synthesize viral DNA, which remains acid insoluble.

The effect of HU on the degradation and reutilization of host DNA was also examined. The degradation and reutilization of host DNA in *dar*1-infected cells were not affected by HU at a concentration as high as 20 mM, where almost no progeny was formed (Table 5).

DISCUSSION

We have isolated mutants defective in a new late gene of bacteriophage T4. This new gene is referred to as *dar* (DNA arrested restoration). *dar* mutants specifically restore the abnormalities (both arrested DNA synthesis and intracellular growth) caused by gene 59-defective mutants. Previously, we have shown that the arrested DNA synthesis can also be restored by the addition of chloramphenicol at 5 to 12 min after infection or by the addition of extragenic mutations in either gene 55 or gene 33 (27). By measuring the kinetics of DNA synthesis, we found that *dar* mutants completely restored DNA synthesis to the wild-type level and the synthesis lasted over 50 min after infection (Fig. 1). However, in the case of suppression by chloramphenicol or extragenic mutations in gene 55 or 33, DNA synthesis is restored only to about 90% of the wild-type level and only lasts for about 30 min (27). Since late genes are not expressed in these cases, the incomplete restoration of DNA synthesis implies that, besides *dar*, other late genes must also be involved in

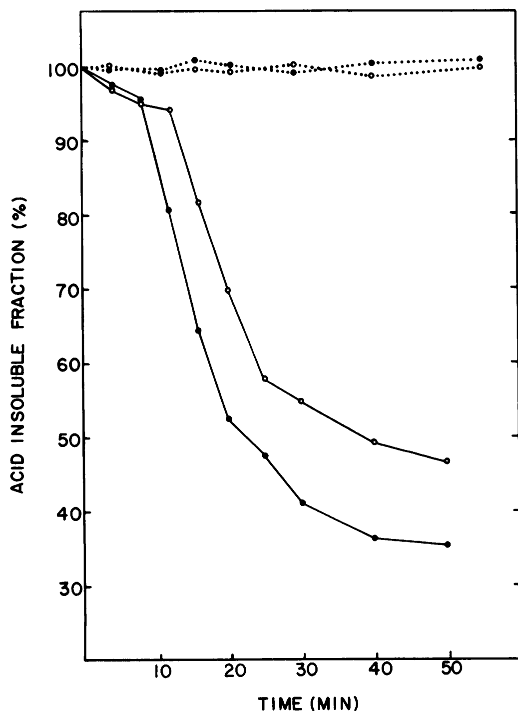

FIG. 7. *Degradation and reutilization of host DNA in dar1-infected cells. E. coli B Tr201 was labeled with [^{3}H]thymine (20μCi/1μg per ml) for two generations, washed twice to eliminate free [^{3}H]thymine before infection, and then infected with phage at an MOI = 8. In the case of amN122 and dar1-amN122, the infected culture contained a high concentration of cold thymine (1,000 μg/ml). In the case of T4D and dar1, the concentration of cold thymine was 1 μg/ml. At various times after infection, samples were taken and radioactivity of the acid-insoluble material was measured. Those sampled at 0 min were considered as 100%. Others were calculated to corresponding percentages. Symbols: —●—, amN122; —O—, dar1-amN122; --●--, T4D; --O--, dar1.*

DNA replication. One of them is gene 49. The function of gene 49 is reported to be required for the completion of packing phage DNA into phage head (14-16). By sedimentation of DNA replicative intermediates in neutral sucrose density gradients, Frankel et al. (6) reported that gene 49 appears to control an endonuclease either directly or indirectly and, when functional, produces a normal 200*S* DNA replicative intermediate in infected cells. Other late endonucleases whose genetic locations are not yet known were reported by Altman and Meselson (1) and Kemper and Hurwitz (13).

Our result shows that *dar* is a late gene (Fig. 4). This conclusion is based on the fact that the function of gene 55 controls the genetic expression of late proteins. However, regardless of the control of late gene expression, if a defect in gene 55 causes a direct effect on the restoration of arrested DNA synthesis, our conclusion would be invalid. In other words, *dar* could be an early gene under this condition. However, this possibility seems unlikely for the following reasons. (i) The only function of gene 55 known thus far is that it controls the genetic expression of late proteins. (ii) Arrested DNA synthesis is also restored by introducing an extragenic mutation in gene 33, which controls the expression of late genes by the same mechanism as gene 55 (11). (iii) Arrested DNA synthesis is restored by the addition of chloramphenicol 5 to 12 min after infection at 37 C (27). By this time, all early genes are expressed, including gene 55. If *dar* is an early gene, DNA synthesis would not be restored under this condition. (iv) Even though the initiation of DNA synthesis appears to be normal in *dar*-infected cells (Fig. 1), it has been shown that DNA replicative intermediates are abnormal in molecular size with respect to wild type (J. R. Wu and Y. C. Yeh, unpublished data). This abnormality starts after 7 min at 37 C and indicates that *dar* function is involved in the late rather than early stage.

dar mutants appear to be HU sensitive (Table 5). Since the primary effect of HU is thought to be the inhibition of the ribonucleotide reductase systems (30; Y. C. Yeh, unpublished data), degraded products of host DNA are the only source of deoxynucleotides for synthesizing viral DNA in the presence of HU. Therefore, the HU sensitivity of a number of mutants is due to a defect in the degradation of host DNA (9, 25). Although *dar* mutants slightly affect the degradation of host DNA (Fig. 7), this effect is too small to account for their HU sensitivity. Other mechanisms were also reported by Goscin et al. (8), such as a defect in the packing of phage DNA into phage particles or in the lysing process of infected cells. It has been shown that HU causes both single-stranded nicks and double-stranded cuts on DNA replicative intermediates in *dar*-infected cells (J. R. Wu and Y. C. Yeh, unpublished data). This effect may account for its HU sensitivity, although HU might also inhibit packing steps or other steps in *dar*-infected cells.

dar mutants have been mapped between genes 24 and 25, contiguous to late genes determining the structure and assembly of the phage head and tail. Other mutants known to map in this region are *y*, *sus*B, and *m*22. These mutants have different phenotypes. Mutant *y*,

which was mapped 7.6 units counterclockwise from *am*N67 (gene 25) (20), was reported to be UV sensitive and to cause a reduction in genetic recombination (2) and a lethal effect on growth (20). *m*22 is similar to *y* in its effect on UV sensitivity and genetic recombination but results in sensitivity to HU and minute plaque morphology (N. V. Hamlett and H. Berger, Cold Spring Harbor Phage Meet., p. 24, 1973). *sus*B, which specifically suppresses gene 49-defective mutants, was reported to be an early, nonlethal mutation (M. Dewey, Cold Spring Harbor Phage Meet., p. 23, 1972; F. R. Frankel, personal communication). *dar*, which specifically suppresses gene 59-defective mutants, is a nonlethal late gene (Table 2), is insensitive to UV irradiation (Fig. 5 and 6), has no effect on genetic recombination (Table 3), makes a large plaque on *E. coli* B, and is sensitive to HU (Table 5). Since these mutants were mapped by different methods, it is hard to judge whether they are closely located by apparent recombination frequency observed by different authors. From the phenotypes, it is likely that these mutants are located in different cistrons.

From the facts that *dar* is mapped far away from gene 59 and does not suppress *amber* mutation in the other 11 genes investigated, it is clear the suppressor of *dar* belongs to the indirect suppressor type classified by Gorini and Beckwith (7). This indirect suppression could be explained at least in two ways. (i) The conformation and function of mutated *dar* protein is slightly changed with respect to the wild type. This modified product may substitute directly for the gene 59 protein. (ii) The mutated *dar* product completely loses its normal physiological function, and thus an alternate or altered metabolic pathway for DNA synthesis is created in *dar-am*C5-infected cells.

If the former alternative accounts for the mechanism of suppression, one would expect that mutant alleles would be trans-dominant to the wild-type allele and that only a few *dar* mutants mutated at such a critical point(s) would be able to suppress mutants defective in gene 59. This alternative is ruled out by the fact that *dar* mutant alleles are recessive to the wild-type allele (Fig. 3).

The working hypothesis for the second alternative might be explained by participation of host function. However, this possibility is unlikely. We have found that *dar* mutants do not restore the UV sensitivity and the rate of replicative repair but partially suppresses the premature release of DNA from the replicative complex (unpublished data). The fact that DNA is prematurely released from the replicative complex of gene 59-defective mutants (29) implies that arrested DNA synthesis might result from damage of replicative DNA on the cell membrane. The result of kinetic studies supports this assumption (29). The result that *dar* suppresses the premature release of DNA from the replicative complex but not other abnormal phenotypes seems to suggest that host function is not involved in the suppression mechanism. It is likely that, by mutation in gene 59. active *dar* protein causes, directly or indirectly, a damage to replicative DNA or other components required for normal DNA synthesis, resulting in an arrest of DNA synthesis. A mutation in *dar* removes this damaging factor, and thus replicative components stay intact in the absence of active gene 59 protein. The integrity of the replicative components causes DNA to be continually synthesized.

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