Gene Expression and Stability of mRNA Affected by DNA-Arrested Synthesis in Gene 59, 46, and 47 Mutants of Bacteriophage T4

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The effect of bacteriophage T4 gene 59 mutations (DNA-arrested synthesis) on kinetics of DNA synthesis, gene expression, and stability of mRNA has been studied. When *Escherichia coli* B was infected by a T4 gene 59 mutant, DNA synthesis proceeded to increase linearly after initiation, but started to decrease at 8 min and was completely arrested at 12 min at 37°C. At various incubation temperatures (20 to 42°C), the initial rates and times of arrest of DNA synthesis were different, but the total amount of DNA synthesized was constant. This result supports the hypothesis that function of gene 59 is required for the conversion of 63S DNA molecules to other replicative intermediates (39). The abnormality in protein synthesis caused by gene 59 mutation is manifested by (i) a delayed shutoff in the expression of early proteins (gene 43, 46, 39, 52, 63, 42-45, and some unidentified proteins), (ii) a reduced rate of late gene expression (gene 34, 37, 18, 20, 23, *wac*, 24, 22, 38, and 19), and (iii) an absence of cleavage of certain late proteins (23, 24, IPIII and 22 to 23*, 24*, IPIII*, and small fragments). It appears that there was no effect on the expression of gene 33, 55, and 32 by a mutation in gene 59. Results obtained from an addition of rifampin at the prereplicative cycle after infection indicated that mRNA from genes 43, *rIIA*, 46, 39, 52, and 63 are more stable in T4 Δ 59 than in wild-type-infected cells. mRNA remained functional longer in mutant-infected cells, and this may explain the prolonged synthesis of certain early proteins. The gene expression of other DNA arrested mutants—those in genes 46 and 47—showed a pattern of abnormal protein synthesis similar to that found in gene 59 mutant-infected cells, except more late proteins are synthesized. The gene expression in terms of phage DNA structure is discussed.

The temporal sequence of bacteriophage T4 gene expression during the development of the T4 phage in its host appears to be precisely regulated (9, 11, 21, 32). For example, expression of prereplicative cistrons, such as immediate and delayed early genes, is controlled by two inter-related mechanisms. The early genes are expressed immediately after infection as the host RNA polymerase recognizes their promoter loci, whereas the delayed early genes are expressed after the read-through of the immediate early gene termination sites, which is caused by the appearance of a T4-specific antiterminator and/or the new initiation of quasi-late promoters (6, 11, 20).

We have studied the role of gene 59 in DNA synthesis and repair. We have shown that the mutation of gene 59 causes arrested DNA synthesis, premature release of replicative DNA

from the replicative complex, little or no concatamer formation, slow repair of progeny DNA fragments, and defective repair of UV and alkylating agent-damaged DNA (35, 36, 39-41). Genes 46 and 47 also belong to the DNA-arrested synthesis (DA) gene class (9, 27). The effect that abnormal DNA synthesis, resulting from mutations of genes in the DA gene class, has on the gene expression of both early and late proteins has not been investigated. The study of gene 59 is, in part, important because it has been mapped between genes 32 and 33, the former of which codes for DNA-binding protein and the latter of which controls late gene expression. Whether or not the gene 59 mutation affects the expression of these genes is not known and requires further study.

In this paper, we present data on the kinetics of DNA synthesis at various temperatures, the abnormal regulation of early and late gene expression, cleavage of late proteins, and stabil-

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ity of mRNA in gene 59 mutant-infected cells. Protein synthesis in gene 59 mutant-infected cells has also been compared with that of gene 46 and 47 mutant-infected cells.

MATERIALS AND METHODS

Bacteria. *Escherichia coli* K strain CR63 (30), which carries an *suI* gene, was used as a permissive host for preparing stocks of T4 *amber* mutants. *E. coli* B strain O21 carrying an *su⁻* gene was obtained from I. Tessman and used in ^{14}C -labeled amino acid labeling of T4 proteins for sodium dodecyl sulfate (SDS) slab-gel electrophoresis.

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 [^3H]-thymine incorporation experiments (B. Dale, Ph.D. thesis, University of Michigan, Ann Arbor, 1968).

Bacteriophage. Bacteriophage T4D was used as wild-type phage in all of the experiments. *amC5*, an *amber* mutant in gene 59, was obtained from R. S. Edgar. It was purified by back-crossing five times with wild-type T4D. *dar* mutants were isolated from our laboratory (36). All other T4 mutants used here were obtained through G. R. Greenberg.

Media. M9 medium (16) was modified and used for growing *E. coli* BO21 in ^{14}C -labeled amino acid labeling experiments. One liter of M9 medium contained: 7 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 3 g of KH_2PO_4 (anhydride), 0.5 g of NaCl, 1 g of NH_4Cl , 24.7 mg of MgSO_4 , 14.7 mg of CaCl_2 , and 4 g of glucose.

Chemicals. A ^{14}C -labeled amino acid mixture was purchased from New England Nuclear Corp. (25 mCi/matam). Acrylamide, *N,N'*-bismethylene acrylamide, and No-Screen medical X-ray film (Kodak) were purchased from Eastman Chemical Co. SDS was obtained from BDH Chemicals Ltd. Other chemicals were standard reagent grade products.

Measurement of kinetics of DNA synthesis. The procedure of measuring the kinetics of DNA synthesis has been described previously (40).

Preparation of ^{14}C -labeled T4 phage proteins for SDS slab-gel electrophoresis. The overnight culture of *E. coli* BO21 was diluted 50-fold with M9 medium and grown to a cell density of 5×10^8 cells per ml with vigorous aeration at 37°C , then D,L-tryptophan was added to 20 $\mu\text{g}/\text{ml}$, and the culture was infected with purified T4 phage particles at a multiplicity of infection (MOI) of 10. At the indicated time, in Fig. 2 and 3, 1.5-ml portions of infected cells were exposed to the ^{14}C -labeled amino acid mixture (0.5 μCi [2 μg]/ml, algal protein hydrolysate, New England Nuclear Corp.) for various time intervals. Incorporation of ^{14}C -labeled amino acids was terminated by pouring the incubation mixture into three volumes of chilled 5% Casamino Acids (Difco). The infected cells were collected by centrifugation, washed with 50 mM Tris-hydrochloride (pH 6.8), and then centrifuged again. The washed cell pellet was resuspended in 0.5 ml of sample buffer, which contained 0.0625 M Tris-hydrochloride (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.01% bromophenol blue. The phage proteins were completely dissociated and denatured by immersing the ^{14}C -labeled sample in boiling

water for 1.5 min. The samples were loaded on the slab gel for immediate electrophoresis or stored at -20°C . Typically, 30,000 cpm in less than 15 μl of each sample were loaded.

SDS slab-gel electrophoresis and autoradiography. The ^{14}C -labeled protein samples were run on the vertical slab-gel system described by Studier (29) by using the SDS gel and buffer system of Laemmli (18) with slight modifications. No-Screen medical X-ray film was exposed to the dried slab-gel by direct contact for various periods of time (normally 4 days). The identification of T4 gene products on the autoradiogram was accomplished by comparison with other published data (21, 31, 38). Therefore, most of the band identifications are tentative or only probable.

RESULTS

Kinetics of DNA synthesis at different temperatures. The synthesis of new viral DNA, which is controlled by the phage itself, is initiated approximately 5 min after infection and continues through the rest of the replicative cycle (9). The arrest of DNA synthesis in cells infected with *amber* mutants of gene 59 begins at 8 min after infection at 37°C and ends at 12 min (39, 41).

Phage DNA synthesis was measured at temperatures between 20 and 42°C by adding [^3H]-thymine at 3 min after infection. Radioactivity in acid-insoluble material obtained at various temperatures was counted (Fig. 1). The time of phage DNA initiation and rate of phage DNA synthesis were studied as a function of temperature; higher temperature resulted in earlier initiation and higher rate of DNA synthesis. The initiation of phage DNA synthesis occurred 3 min after infection at 42°C , 6 min at 30°C , and 15 min at 20°C . After initiation, DNA synthesis proceeded at different rates at different temperatures. In T4*amC5*-infected cells, the time of arrest of DNA synthesis, as well as the total amount of DNA synthesis, varies with the temperature, but the total amount of [^3H]thymine incorporation (about 10^4 cpm per 5×10^7 infected cells or 0.5 μg of DNA per 5×10^7 infected cells) remains constant in spite of temperature variation. The phage DNA synthesized, as determined by diphenylamine, was equivalent to 10^{-8} μg of phage DNA synthesized per infected cells. This amount is equivalent to approximately 50 phage equivalents of DNA synthesized for one phage-infected bacterium, when the molecular weight of the T4 DNA molecule is taken to be 123×10^6 daltons (17). These results show that DNA replication of T4 gene 59 mutants has normal initiation of DNA synthesis, proceeds to some definite stage, and then ceases.

Effect of gene 59 mutation on T4 gene expression. The effect of gene 59 mutation on the expression of phage T4 proteins was moni-

FIG. 1. Kinetics of DNA synthesis in T4D- and T4amC5 (gene 59)-infected cells at various temperatures. A culture of *E. coli* B Tr201, at a concentration of 5×10^8 cells per ml, was infected with T4D or T4amC5 (MOI of 5) with aeration at different temperatures. [^3H]thymine ($20 \mu\text{Ci}$ [$3 \mu\text{g}$]/ml) was added at 3 min (42, 37, 30, and 25°C) or 6 min (20°C) after infection. Samples of 0.1 ml were withdrawn at various intervals to measure radioactivity in the acid-insoluble fraction as described in the text. Symbols: T4D: $\square$ — $\square$, 42°C; $\bullet$ — $\bullet$, 37°C; $\circ$ — $\circ$, 30°C; $\triangle$ — $\triangle$, 25°C; ∇ — ∇ , 20°C; T4amC5: $\square$ — $\square$, 42°C; $\bullet$ — $\bullet$, 37°C; $\circ$ — $\circ$, 30°C; $\triangle$ — $\triangle$, 25°C; ∇ — ∇ , 20°C.

tored by autoradiography of SDS polyacrylamide slab gels after electrophoresis of ^{14}C -labeled amino acid-labeled viral proteins. This procedure provides more specific information than that of RNA-DNA hybridization, which can only distinguish a few classes of mRNA expressed. Either T4D or T4amC5-infected cells were pulse labeled with ^{14}C -labeled amino acids at the time indicated at 37°C, and the incorporation rates were determined. The rate of incorporation of ^{14}C -labeled amino acids gradually increased after infection, reaching the maximum

rate at 10 to 12 min postinfection, and then decreased (Table 1). The incorporation rates in both T4D- and T4amC5-infected cells were almost identical up to 30 min postinfection. The ^{14}C -labeled amino acid-labeled proteins from infected cells were then subjected to SDS slab-gel electrophoresis after complete lysis in 2% SDS. Identical amounts of ^{14}C -labeled proteins were layered onto each gel slot for electrophoresis. The differences in intensity of each protein on autoradiograms should reflect the difference in relative rate or amount of protein synthesized.

TABLE 1. Rate of ^{14}C -labeled amino acid incorporation in T4D- or T4amC5-infected *E. coli* BO21 cells^a

Labeling time (min postinfection)	^{14}C -labeled amino acid incorporation (cpm/ 7.5×10^7 cells)	
	T4D	T4amC5
2-4	34,800	36,500
4-6	35,800	40,000
6-8	40,900	40,500
8-10	41,300	40,600
10-12	41,700	41,600
12-14	44,500	41,200
14-16	40,600	39,000
17-20	38,700	38,800
22-25	28,700	27,700
27-30	24,000	21,350

^a *E. coli* BO21 cultures (5×10^8 cells per ml) were grown in M9 medium and infected at 37°C with phage at an MOI of 10. At the time indicated, the T4-infected cells were exposed to a ^{14}C -labeled amino acid mixture ($0.5 \mu\text{Ci}$ [$2 \mu\text{g}$]/ml). Under this condition the incorporation of ^{14}C -labeled amino acids remained linear for at least 4 min. The infected cells were washed free of ^{14}C -labeled amino acids by centrifugation and lysed in 0.1 ml of sample buffer by heating at 100°C for 2 min. A 10- μl portion of each lysate was placed onto round Whatman filter paper (no. 3, 2.5-cm diameter) to determine the radioactivity in trichloroacetic acid-insoluble fraction. The same samples were subjected to SDS slab-gel electrophoresis (Fig. 2). Note that the last three pulses are for 3 min, whereas the rest are for 2 min.

The effect of the gene 59 mutation on gene expression of early and late proteins and the conversion of gene products has been examined and is shown in Fig. 2. The expression of most of the immediate early and delayed early proteins, which are synthesized before the onset of DNA synthesis, ceased at 10 to 12 min postinfection at 37°C in wild-type T4D-infected cells. However, in T4amC5-infected cells, the shutoff of early protein synthesis was delayed, and synthesis of some of these proteins was prolonged even until 30 min postinfection. These early proteins included genes 43, 46, 39, 52, 63, 42-45, and some other unidentified phage proteins. However, other early proteins were not affected, such as *rIIA*, 32-44, *rIIB*, and *IP III*. In wild type, the late proteins were expressed at 8 min postinfection under our conditions, as judged by autoradiogram (Fig. 2). All of the late proteins were synthesized in T4amC5-infected cells, but the rate of synthesis of some late proteins was lower (e.g., gene proteins 34, 37, 18, 20, 23*, *wac*, 24, 22, 38, and 19) when compared with that of wild-type-infected cells. The rate of synthesis of other late proteins in T4amC5-infected cells was apparently the same as that of wild-type-infected cells; e.g., gene proteins 7, 10, 8, and 35. Four head-associated phage proteins, such as those of genes 22, 23, 24, and *IP III*, are cleaved to smaller polypeptides, namely, 23*, 24*, and *IP III**, during morphogenesis (18). Gene 22 product is

FIG. 2. Autoradiogram of SDS slab-gel after electrophoresis of protein pulse labeled with ^{14}C -labeled amino acids at the time indicated after infection of *E. coli* BO21 by T4D or T4amC5. See text for detailed information. Gene products of early and late genes are indicated on both sides on the plate. Symbols: ●, T4D; ○, T4amC5.

cleaved to form an unidentified product (13, 18). The conversion of 23 to 23* is very inefficient and that of IPIII to IPIII* did not occur in T4amC5-infected cells (Fig. 2). The conversion of 24 to 24* was not resolved under these experimental conditions. The available evidence suggests that the proteolytic cleavages of head-associated phage proteins are intimately linked to the head assembly process (19). The cleavage of the products of genes 23, 24, IPIII, and 22 to 23*, 24*, IPIII*, and small fragments is blocked by mutation in any of the head genes 20, 21, 22, 23, 24, and 31. Onorato and Showe have given evidence that proteolytic activity resides in the gene 21 protein (22). However, T4-specific proteolytic activities are found at 8 to 10 min and are maximal at 18 min after infection (2, 23). The expressions of the proteolytic enzyme(s) may be reduced by the gene 59 mutation, and, in consequence, the proteolytic cleavage of a number of proteins is reduced by gene 59 mutation. Another possible reason for the lack of head protein cleavage may be the abnormal DNA synthesis in this mutant, since complete head assembly normally depends on DNA packaging events (19). Since the early genes 33 and 55 control the expression of late genes (3, 24, 28) and since much late protein is made by a gene 59 *amber* mutant (Fig. 2), it appears that expression of genes 33 and 55 is not seriously affected (if at all) by a mutation in gene 59.

Stability of early mRNA's in gene 59 mutant-infected cells. Some immediate early genes (genes 39 and 52) and delayed early genes (genes 43, 46, and 42-45) in *amC5* (gene 59)-infected cells showed prolonged expression (Fig. 2). Possible reasons for this delay in shutoff of early genes are an increased rate of early mRNA synthesis, an increased stability of early mRNA (or slower rate of mRNA degradation), or both. These alternatives can be distinguished by treatment of the infected cells with rifampin. Rifampin binds to the β -subunit of the *E. coli* RNA polymerase and thereby inhibits the initiation of transcription (12). If the gene 59 mutation affects early RNA synthesis, the gene expression of mutant-infected cells after rifampin treatment at the early stages of infection should be the same as the wild-type-infected cells because the effect of gene 59 function of gene expression occurs after 10 min of infection. On the other hand, if the early messenger of mutant-infected cells remains functional longer than that of wild-type-infected cells, the early gene shutoff in mutant-infected cells will be prolonged even after rifampin treatment.

E. coli BO21 cells were infected by T4D or T4amC5 at 37°C (MOI, 10), and rifampin was

added to a final concentration of 400 μ g/ml at 4 min after infection to inhibit early mRNA synthesis. The capacity of rifampin-treated cells to synthesize early proteins was measured by pulse-labeling with 14 C-labeled amino acids followed by SDS slab-gel electrophoresis (Fig. 3). In the rifampin-treated cells, the same amount of mRNA was synthesized in T4D- and T4amC5-infected cells, as indicated by the fact that both T4D and T4amC5 have the same pattern of gene expression for the first 10 min after phage infection. However, some early proteins such as gene proteins 43, *rIIA*, 46, 39, 52, and 63 were synthesized more in T4amC5- than in wild-type-infected cells. This is consistent with the greater stability of the mRNA of those genes in T4amC5 infection than in T4D. The messengers of other early genes such as (32-44-*rIIB*) and IPIII apparently have the same stability in T4D- and T4amC5-infected cells, as indicated by the identical intensity of the protein bands in the autoradiograms (Fig. 3). Since the effect of the gene 59 mutation on the expression of some early genes is due to this effect on the stability of mRNA rather than on RNA synthesis, this indicates that gene 59 may act on phage gene expression by affecting some nuclease activity related to the degradation of these early mRNA's. This gene 59-related nuclease activity appears to be specific for gene 43, *rIIA*, 46, 39, 52, and 63 mRNA's, but not those for (32-44-*rIIB*) and IPIII. This specificity of mRNA degradation may be due to different nucleases acting on these two groups of early mRNA's. Other possible reasons for the delay in shutoff are presented in Discussion.

Temporal relationship between expression of gene 59 protein and its effect on gene expression in T4 phage infection. Normal initiation of DNA synthesis in T4amC5-infected cells occurs, but the rate of DNA synthesis begins to decrease at 8 min and completely arrests at 12 min (41). Since the gene 59 mutant-infected cells show delayed shutoff of early genes and expression of late genes, the question can be asked: at what time after phage infection does gene 59 begin to exert its effect on phage gene expression? This question was examined by adding rifampin at various times after T4D infection and labeling phage proteins with 14 C-labeled amino acid at the late lytic cycle (12 to 18 min after infection). In contrast to the above experiment (Fig. 3), we focused on late gene expression. When rifampin was added up to 6 min after wild-type T4D infection to block further initiation of mRNA synthesis, the T4D-infected cells only synthesized prereplicative gene products but showed no late gene expression. This result

FIG. 3. Decay of protein-synthesizing activity in T4D- and T4amC5-infected cells after rifampin treatment. *E. coli* BO21 culture was grown in M9 medium to 5×10^8 cells per ml at 37°C and infected with phages at an MOI of 10. At 4 min after infection, rifampin was added to a final concentration of $400 \mu\text{g/ml}$. At the indicated time after infection, ^{14}C -labeled amino acids were added to $0.5 \mu\text{Ci}$ ($2 \mu\text{g}$)/ml of infected cultures. The conditions for electrophoresis and autoradiography are described in the text. Symbols: ●, T4D; ○, T4amC5.

is in contrast to T4amC5-infected cells without rifampin treatment, even though the phage proteins were labeled at the late stage after infection (data not shown). Although early gene expression was affected as early as 4 min, this result shows that the gene 59 product did not exert its effect on late gene expression until 6 min after infection. However, when rifampin was added at 8 min after infection, the pattern of phage protein synthesis was the same as T4amC5-infected cells without rifampin treatment, showing delayed shutoff of early proteins and reduced synthesis of late proteins.

Ten minutes after T4D infection, more late proteins were gradually synthesized than in T4amC5-infected cells without rifampin treatment. The conclusion from this experiment is that gene 59 starts to exert its effect on gene expression at 8 min after infection. The time that gene 59 function starts to act on phage

expression coincides with the arrest of DNA synthesis (41).

Comparison of gene expression of gene 59 mutants with other DNA arrest mutants (genes 46 and 47). The gene expression of T4amC5 was compared with other DA mutants: T4amN130 (gene 46) and T4amA456 (gene 47). Early gene expression was the same as in T4amC5 (gene 59)-, T4am130 (gene 46)-, T4amC5-amN130 (gene 59-46)-, T4am130-amA456 (gene 46-47)-, and T4amC5-amN130-amA456 (gene 59-46-47)-infected cells (data not shown). All of these DA mutant-infected cells showed delayed shutoff of early genes when compared with wild-type infected cells. However, some differences were observed in late protein synthesis between gene 59 mutant and gene 46, 47 mutant-infected cells. In a late stage after phage infection (20 to 22.5 min), some late protein products such as 34, 37, and 23* were

synthesized more in T4amN130- and T4amN130-amA456-infected cells than in T4amC5-, T4amC5-amN130- and T4amC5-amN130-amA456-infected cells.

Effect of *dar* mutation on gene expression of gene 59 mutant. We have isolated *dar* mutants (DA restoration) which are extragenic suppressors of the gene 59 mutant (36). These mutations suppress the defect in DNA synthesis of DA mutants of genes 46, 47, and 59. In addition, progeny formation by gene 59 mutants is restored by a *dar* mutation. We examined the effect of a *dar* mutation on the gene expression of the gene 59 mutant by constructing a double mutant, T4dar1-amC5 (*dar*-gene 59) (data not shown). We found in T4dar1-amC5-infected cells that the shutoff of known early genes such as 43, 46, 39, and 42-45 was delayed up to 18 min after infection, but the time of shutoff was still earlier than that of T4amC5-infected cells.

Although the temporal expression of late proteins in dar1-amC5-infected cells was similar to that in T4dar1-infected cells, most of the late proteins and the conversion of head proteins (23 to 23*, 24 to 24*, and IPIII to IPIII*) were reduced. These results indicated that although T4dar1 restored the arrested DNA synthesis and progeny formation of gene 59 mutation to a normal level, the gene expression of T4dar1-amC5 was not as complete as that of the T4dar1 control. The burst size of T4dar1-amC5 was increased to 200 ± 10 , as compared to 25 ± 5 in T4amC5-infected cells. We have also compared gene expression of T4dar1 with that of T4D (37).

Expression of gene 32 protein (DNA binding) in gene 59 mutant-infected cells. In dar-amC5 (*dar*-gene 59) double mutant-infected cells, we found overproduction of gene 32 protein (DNA binding) as compared with amC5 (37). Gene 32 protein is known to be involved in DNA replication, repair, and recombination (1, 41), and gene 32 is contiguous to gene 59 in the genetic map. However, in the present study the expression of gene 32 protein in gene 59 mutant-infected cells was normal (data not shown), indicating lack of a polar effect.

DISCUSSION

At various incubation temperatures, the total DNA synthesized in the gene 59 mutant-infected cells was equivalent to about 50 phage DNA units per infected bacterium. This constant amount of DNA synthesis, occurring at various initial replication rates, indicates that mutant DNA replication proceeds to some fixed point and then stops. The arrest time of DNA replication in this mutant is the same as that for the

appearance of defective joining of progeny DNA and premature release of replicative DNA from cativ complex (41). This suggests that gene 59 is involved in the conversion of early replicative intermediates to late ones, which is characterized by the formation of concatenated molecules from monomers of DNA.

We have also shown in this paper that a mutation in gene 59 has a pleiotropic effect on T4 phage gene expression, causing delayed shutoff of early genes, reduced expression of late genes, and blockage of head-associated protein conversion. In addition, some early mRNA's remained functional longer in a gene 59 mutant than in wild-type-infected cells. This result suggests that some RNase activity involved in the degradation of early mRNA is affected by gene 59 mutation.

Some possible explanations for delayed shutoff of some immediate and delayed early genes are as follows. (i) The early mRNA's are continuously synthesized in gene 59 mutant-infected cells, and the pattern of phage protein synthesis is simply due to the competition between early and late mRNA's for the translation apparatus. When rifampin is added at the early stage after phage infection to block the initiation of transcription, the early protein synthesis in the mutant still continues longer than in the wild-type (Fig. 3). This model assumes the same rate of mRNA synthesis, but says that the gene 59 mutant makes it longer period of time. Another variation of this model says that the gene 59 mutant could make early mRNA faster from time zero. (ii) The gene 59 product is responsible for a modification (such as methylation) of the existing early messengers, rendering them non-translatable. This function cannot easily explain the requirement for the gene 59 function in T4 DNA replication and DNA repair (30). (iii) By mutation in gene 59, the stability of some early gene messengers is increased (Fig. 3). Thus, gene 59 product may contribute an RNase activity which is responsible for the degradation of some early messengers at a certain time after infection. This kind of activity is consistent with the pleiotropic effects of this gene mutation. (iv) There is a greater initial rate of synthesis of early mRNA or delayed shutoff of early transcription; if ribosomes are normally saturated with mRNA, this extra mRNA will have little effect on protein synthesis until mRNA decays below saturating levels. Then protein synthesis should last longer in the gene 59 mutant case (its higher level would take longer to decay below saturation), without invoking any change in the inherent decay rate. (v) Some translational shutoff of early protein synthesis may be dimin-

ished by the gene 59 mutant state. This could indirectly extend the lives of the affected mRNA by protecting them with ribosomes, as seems to be the case with *regA* translational control (14, 34).

The control of the shutoff of early genes by gene 59 is different from that in mutants T4SP62 (33, 34) and T4R9 (14), which can prolong early gene expression up to 60 min compared to 12 min for the gene 59 mutant. Both T4SP62 and R9 are mutated in *regA* gene, which maps between genes 43 and 62 in the T4 genome (14, 33). The abnormalities of early gene expression of SP62 and R9 mutants can be detected only in DNA negative (DO) or maturation defective (MD) states. Mutations in DO or MD genes can cause a delay in early gene shutoff of about 12 min (14). Thus, it was thought that the first stage (S1) was that controlled by DO or MD genes, and the second stage (S2) was that controlled by *regA* (34). The delayed shutoff of early genes in DO or MD mutants may be due to lack of a late protein that is required either for the switch of translation from early to late messengers or the degradation of early messengers (4, 34), but it could equally be due directly to lack of competition from late mRNA, which is not made in DO or MD infections. Although the late proteins are expressed in the gene 59 mutant, the shutoff of early proteins is still delayed for 12 min (Fig. 2 and 3). The shutoff of early messenger synthesis is delayed by addition of chloramphenicol to block early protein synthesis, indicating that some early proteins are involved in the shutoff mechanism (5). At least the gene 59 function, an early gene function, may also participate in the shutoff mechanism for early proteins either directly or indirectly. This may be an exception to the postulated first-stage shutoff mechanism for early protein(s) that is solely controlled by late protein(s) (5).

It has been shown by using rifampin that the delayed shutoff of early proteins by the T4R9 mutation has a correlation with increased stability (14, 33). The increased stability could be a secondary result of continued selection of those mRNA by ribosomes (14, 15, 34). The T4R9 mutation prolonged the expression of some early proteins, such as *rIIA*, (32-44-*rIIB*), and 42-45, but not 43, 39, 53, and IPIII (14). On the other hand, T4amC5-infected cells showed prolonged expression of early genes, such as 43, 46, 39, 52, 63, and 42-45, but not *rIIA*, (32-44-*rIIB*), and IPIII (Fig. 2). It appears that gene 59 and *regA* express their effects through different mechanisms to act on early gene shutoff: e.g., the specificities of gene 59 and *regA* functions are different with regard to degradation of the early messengers.

In DO or MD mutant-infected cells, no late

genes are expressed (3, 28, 34), whereas the gene 59 mutant can express some late proteins. The difference in late gene expression between gene 59 mutants and DO or MD mutants is probably due to the fact that gene 59 mutant-infected cells have normal initiation of DNA synthesis for 3 min (35) and the assumption that it has normal phage modification of host RNA polymerase. The progeny DNA molecules that are synthesized before arrest can be transcribed for some late gene expression. However, this small amount of late protein synthesis by the gene 59 mutant can be eliminated by the introduction of a DO or MD mutation (data not shown). These results indicate that replicating DNA can be used for late gene expression and parental DNA is required for early gene expression. T4 parental DNA can be used as a template for late gene expression when a triple mutant, which is defective in DNA polymerase (gene 43), DNA ligase (gene 30), and exonuclease (gene 46) is used to infect a nonpermissive host (8, 38). This requirement of a multiple mutation for replication-independent expression of late genes tends to support the concept that normally only the newly replicated phage DNA, which is different from parental, is competent for late gene expression (7, 25). The competent DNA contains gaps or breaks, which are essential for the initiation of late gene expression. Soon after complete arrest of DNA synthesis in gene 59, 46, and 47 mutant-infected cells, the expression of late genes is affected (data not shown). This effect indicates that gene 59, 46, and 47 functions may be directly or indirectly involved in the creation of nicks or gaps necessary for late gene expression, since some progeny DNA is synthesized before DNA arrest, and modification of host RNA polymerase is assumed to be normal. Further studies on abnormalities in the regulation of protein synthesis and the structure of DNA molecules in gene 59-infected cells may provide better understanding of its biochemical function.

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