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Control of Gene Function in Bacteriophage T4

IV. Post-Transcriptional Shutoff of Expression of Early Genes

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The selective and sequential shutoff of synthesis of early T4 proteins in bacteria infected with DNA-negative mutants is under the active control of one or more T4-induced proteins. Selective shutoff of synthesis of early T4 proteins is accompanied by a selective degradation of distinct species of T4 mRNA. We present circumstantial evidence that selective degradation of mRNA is the cause, and not the consequence, of selective termination of expression of early T4 genes. The mutation sp62 inactivates the shutoff mechanism and prevents the selective degradation of distinct species of T4 mRNA.

The expression of early T4 genes, in wild-type T4-infected *Escherichia coli* B, is terminated around the middle of the latent period (1, 6). This termination is prevented when bacteria are preinfected with bacteriophage T3 (17). T4 phage are released under those conditions, and it appears that the shutoff of early genes is not an obligatory step in T4 development. Termination of early T4 protein synthesis may be due to selective shutoff of mRNA synthesis (7, 15, 21), selective degradation of mRNA (14, 20), and/or selective termination of translation of classes of T4 mRNA (9, 13).

In cells infected with DNA-negative mutants of T4 (T4 DO), synthesis of early T4 proteins is shut off in a sequential mode, i.e., synthesis of some proteins is shut off early, synthesis of others late after infection. We have studied the causes of the selective shutoff in T4 DO, and we report here that T4 controls a mechanism which terminates the expression of early genes in a selective and sequential fashion. The shutoff depends upon the synthesis of active proteins after T4 infection. At least one of the proteins required for the shutoff either is the gene product of locus sp62 (J. S. Wiberg, S. Mendelsohn, G. Warner, K. Hercules, and J. Munro Fed. Proc. **30**:1263, 1971) or is under the control of sp62. Selective termination of synthesis of early T4 proteins is accompanied by preferential degradation of certain species of early T4 mRNA.

MATERIALS AND METHODS

Bacteriophage. T4D, T4D sp62x3, and the following mutants of T4, deficient in the synthesis of T4 DNA (DO), were used: T4 43,44 (amB22x5, amNG485); T4 43,44,62 (amB22x5, amN82, amE1140); T4 sp62, 43, 44, 62 (sp62x3, amB22x5, amN82, amE1140). We are grateful to J. S. Wiberg for providing us with most of these mutant phages.

Bacteria. Experiments were carried out with host *E. coli* B_{s-1} (5) and the amino acid-requiring derivatives B_{s-1} *met*$^-$ and B_{s-1} *pro*$^-$, both isolated in our laboratory. For preparing and assaying lysates of T4 amber mutants we used *E. coli* CR63.

Chemicals and radiochemicals. Sodium dodecyl sulfate (SDS) and *N,N'*-methylene-bis-acrylamide (BIS) were purchased from Bio-Rad Laboratories, Richmond, Calif., and acrylamide from Eastman Kodak Co., Rochester, N. Y. The following items from Schwarz/Mann, Orangeburg, N. Y.: rifampin, nalidixic acid, Tris, L-ethionine, and ^{14}C-amino acids, algal profile, at an approximate average specific activity of 300 mCi/mmol, and uridine-5-^3H. Azetidine-2-carboxylic acid was purchased from Calbiochem, San Diego, Calif.

Media. M-9 medium, consisting of KH_2PO_4, 3.0 g; $Na_2HPO_4 \cdot 12\,H_2O$, 15 g; NH_4Cl, 1.0 g; H_2O; 1,000 ml; glucose at 0.4% (wt/wt), and $MgSO_4$ (10^{-3} M), pH 6.9, was used for growth of bacteria and of infective centers. T4 adsorption was carried out in the presence of 2×10^{-5} M tryptophan (2).

Growth temperature for bacteria and infective centers was 37 C. Bacteria were infected at absorbancy measurement at 600 nm (A_{600}) of approximately 0.65 (equals 5 to 6 $\times$ 10^8 bacteria/ml) at a multiplicity of infection of 10 to 12 phages per cell.

Starvation of bacteria for amino acids. B_{s-1} *met*$^-$ was grown in M-9 medium, supplemented with 5 μg of L-methionine per ml, which results in methionine starvation at an A_{600} of 0.67 (Fig. 1). With B_{s-1} *pro*$^-$, 20 μg of L-proline per ml is required to reach starvation at an A_{600} of 0.65. Termination of growth is equally abrupt with methionine- and proline-requiring bacteria, allowing a fairly accurate determination of the onset of starvation.

Inhibition of RNA synthesis. Rifampin (18) was

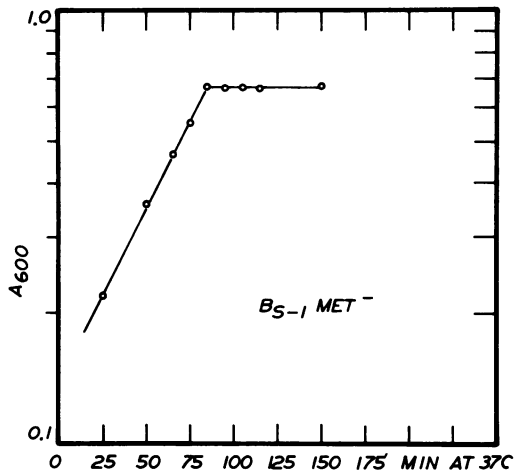

FIG. 1. *Growth curve of E. coli B_{s-1} met⁻ in M-9 medium supplemented with 5 μg of L-methionine per ml. Ordinate, A_{600} of bacterial culture; abscissa, time of incubation at 37 C. Note the well defined termination in increase of absorptivity at the time of methionine depletion of the medium.*

added to a final concentration of 200 μg/ml. Residual uptake of ^{3}H-uridine at 3 min after rifampin addition was less than 5% of normal.

Inhibition of DNA synthesis. Nalidixic acid (11) was added simultaneously with rifampin to a concentration of 10 μg/ml.

Labeling of proteins. Samples (1 ml) of A_{600} = 0.6 cultures were labeled with 2 μCi of ^{14}C-amino acids for times varying from 3 to 5 min. During these pulses there is little depletion of radioactive amino acids. Completion of nascent, radioactive proteins was allowed through a 3-min incubation with a 10^3-fold excess of nonradioactive amino acids. The infective centers were then chilled by addition of 5 ml of cold Tris-chloride buffer (12 g of Tris base, 1,000 ml of H_2O, pH 6.8) and sedimented. The supernatant fluid was removed, and the pellets were resuspended into 0.1 ml of sample buffer (6 g of Tris-chloride, 10 g of SDS, 1,000 ml of H_2O, pH 6.8, plus 10 ml of mercaptoethanol and 150 ml glycerol added) and boiled for 2 to 3 min.

Determination of mRNA half-lives. We used the procedure described by Pato and von Meyenburg (11) for determination of mRNA half-lives.

DNA-RNA hybridization-competition experiments. Radioactive and nonradioactive RNAs were isolated by the CsCl precipitation method (16). Conditions for liquid hybridization-competition were: total volume, 0.4 ml; radioactive RNA, 5 μg/0.4 ml; pH 13 denatured DNA, 4 μg/0.4 ml; competitor RNA as indicated in Fig. 14 and 15; hybridization was carried out for 5 h at 66.5 C in 4.25 × SSC (standard sodium citrate 1X = 0.15 M NaCl, 0.02 M sodium citrate, pH 8.0). Unhybridized RNA was digested by 15 μg of RNase per 0.4 ml (Worthington Biochemical Corp., Freehold, N. J., RNase boiled for 15 min at pH 5.0) for 30 min at 36 C. Undigested RNA was precipitated in trichloroacetic acid and filtered onto Schleicher and Schuell B-6 membrane filters, and the radioactivity measured by scintillation counting.

SDS polyacrylamide slab gel electrophoresis. We used the method described by Studier (19) for SDS polyacrylamide gel electrophoresis. Proteins were quantitated by autoradiography and subsequent densitometry of autoradiographs.

RESULTS

Synthesis of early T4 proteins in T4 wild type and T4 DO-infected E. coli B_{s-1}. When *E. coli* B_{s-1} is infected by DO mutants of T4, early T4 genes are expressed to the normal extent, whereas expression of late genes is greatly reduced (1). Synthesis of early gene products decreases with time after infection in a pattern similar to the loss of early gene expression in T4 wild type-infected cells. We show in Fig. 2 autoradiograms of T4 proteins, labeled with ^{14}C-amino acids at various times after infection and separated on SDS acrylamide gels. The right frame displays the proteins synthesized at different times after T4 wild type infection of *E. coli* B_{s-1}. The left frame shows the pattern of protein synthesis observed with T4 DO mutants. In both cases, synthesis of early T4

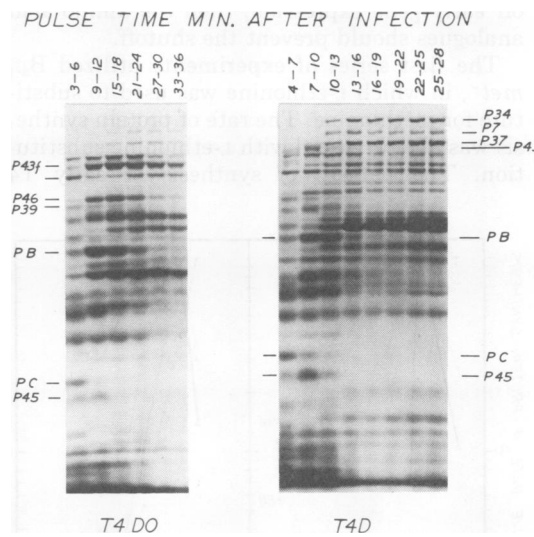

FIG. 2. *Autoradiographs of ^{14}C-amino acid-labeled T4 proteins synthesized at various times after infection of E. coli B_{s-1}. Left, T4 43,44-infected cells; right, T4 wild type-infected cells. Temperature of incubation, 37 C. A 2-μCi amount of ^{14}C-amino acids was added to 1.0 ml of 5 × 10^8 infective centers for the times indicated on the top of the autoradiographs. Rates of synthesis of the proteins indicated on the side of the autoradiographs have been plotted as a function of time in Fig. 3. P34, Gene 34 protein; P43f, amber fragment of gene 43 protein; PB and PC, unidentified bands.*

proteins ceases, but more rapidly with T4 wild type than with T4 DO. It is noteworthy, that those early proteins which are synthesized longer in wild type infections display extended synthesis in T4 DO-infected cells. Figure 3 shows the relative rates of synthesis of proteins P46, PB, PC, and P45 at different times after infection with T4 wild type and with T4 DO. The similar patterns of reduction in early gene expression in T4 DO and in T4 wild type may indicate a common shutoff mechanism.

Shutoff of early protein synthesis in the presence of amino acid analogues in T4 DO-infected cells. To investigate whether the shutoff of early T4 protein synthesis is an active, T4 controlled step, we followed the rates of synthesis of early T4 proteins at various times after infection of *E. coli* B_{s-1} *met*$^-$ and B_{s-1} *pro*$^-$, in the presence and absence of amino acid analogues. Synthesis of active proteins is inhibited, or greatly reduced, by the incorporation of amino acid analogues in the absence of the natural amino acid, as we have shown by the complete inhibition of T4 DNA synthesis in T4 wild type infections of methionine-starved B_{s-1} *met*$^-$ supplemented with L-ethionine (12) (data not shown). If a T4 protein were required to shut off early gene expression, then the amino acid analogues should prevent the shutoff.

The first series of experiments utilized B_{s-1} *met*$^-$, in which L-ethionine was used to substitute for methionine. The rate of protein synthesis was about normal with L-ethionine substitution. The pattern of synthesis of early T4

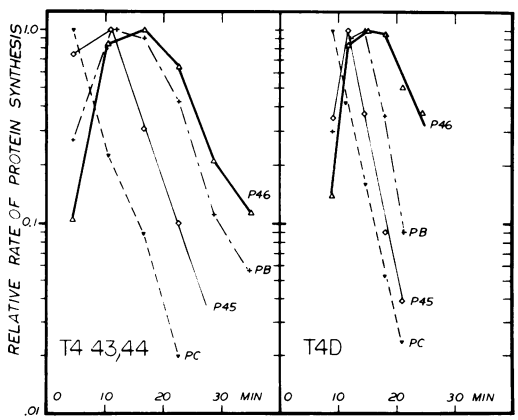

FIG. 3. *Rates of* ^{14}*C-amino acid uptake into early proteins of T4 at various times after infection of E. coli* B_{s-1}. *Left, after infection with T4 43, 44; right, after infection with T4 wild type. Ordinate, Normalized* ^{14}*C-amino acid uptake; abscissa, time after infection at 37 C. Symbols: △, protein 46; +, protein PB; ◇, protein 45; ▽, protein PC (see Fig. 2).*

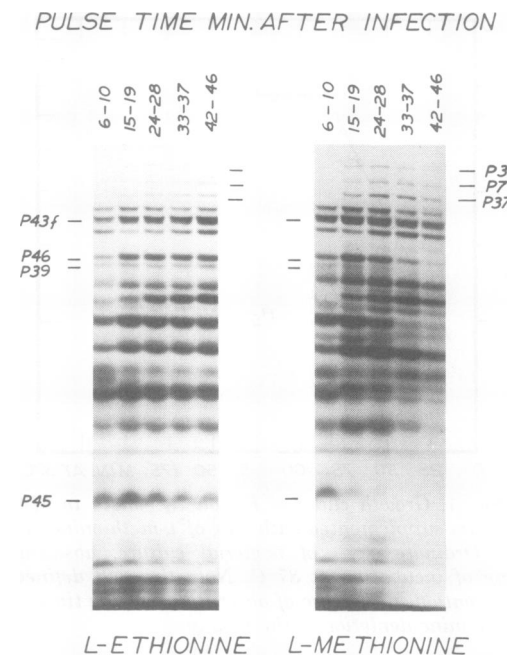

FIG. 4. *Patterns of synthesis of early proteins after infection of E. coli* B_{s-1} *met*$^-$ *with T4 43,44 in the presence and absence of amino acid analogues. Left, In the presence of* L-*ethionine; right, in the presence of* L-*methionine. Times of pulsing with amino acids are indicated on the autoradiographs. Conditions of labeling as in Fig. 2. Experimental scheme:* B_{s-1} *met*$^-$ *was starved for methionine for 30 min by depletion of the 5 μg of supplement of methionine per ml in the M-9 medium (see Fig. 1). T4 DO, at a multiplicity of infection of 10 to 12, was added to bacteria in the cold. To one-half of the culture 10 μg of* L-*methionine per ml was added; to the other half 10 μg of* L-*ethionine per ml was added. The two cultures were incubated at 37 C, and 1.0-ml samples were pulse labeled with a methionine-free mixture of* ^{14}*C-amino acids. The labeled proteins were finally resolved on 10% SDS acrylamide gels.*

proteins at various times after infection by T4 DO is shown in Fig. 4 (left, after substitution by L-ethionine; right, with L-methionine). The shutoff of early protein synthesis is essentially eliminated by the presence of the amino acid analogues.

Since the lack of methionine will affect the intracellular levels of S-adenosylmethionine, and consequently the extent of methylation of nucleic acids and the synthesis of spermidine, we repeated the experiments with a proline-deficient mutant of *E. coli* B_{s-1}. The experimental conditions were identical to the ones described for methionine starvation except that azetidine-2-carboxylic acid was used as a proline analogue (12). Figure 5 shows that azeti-

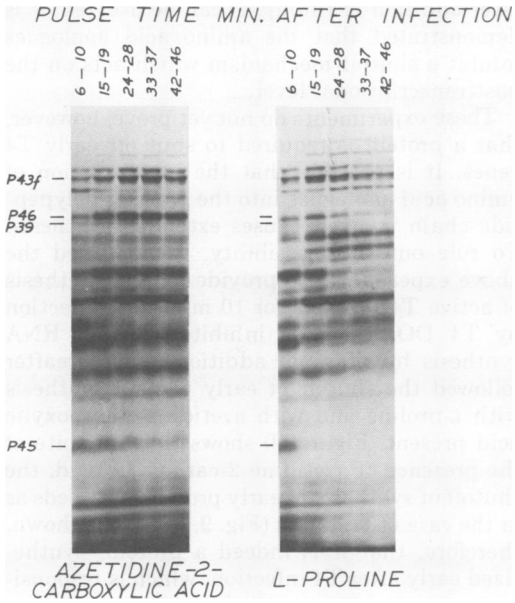

FIG. 5. *Patterns of synthesis of early proteins after infection of B_{s-1} pro⁻ with T4 43,44 in the presence and absence of amino acid analogues. Same experimental conditions as described in Fig. 4 except that cells were starved for proline, and azetidine-2-carboxylic acid (40 μg/ml) was used to substitute for* L-*proline.*

dine-2-carboxylic acid prevents the shutoff of early T4 protein synthesis to about the same extent as L-ethionine.

Attempts to correlate mRNA half-life with the extension of early protein synthesis. It is conceivable that the presence of the amino acid analogues is primarily affecting the half-lives of mRNA and, thus, lead to an extension of early protein synthesis. Half-lives of mRNA were, therefore, determined in the same cultures used for the studies of protein synthesis displayed in Fig. 4 and 5. In both methionine- and proline-starved cells, half-lives of RNA synthesized around 7 min and 34 min after T4 DO infections were measured. The results (Fig. 6 and 7) do not provide an unambiguous correlation between extension of protein synthesis and mRNA half-lives.

In the case of methionine addition to methionine-deficient B_{s-1}, the ^{3}H-uridine uptake at 6 to 7 min after infection occurs at twice the rate observed with L-ethionine addition. The RNA decay curves are bi-phased, and the half-lives of the rapidly degraded fractions of RNA are similar (2.5 and 3 min). The residual, more stable RNA contains about equal amounts of radioactivity (see Fig. 6, top). RNA synthesized at 33 to 34 min after infection displays similar patterns, except that the fraction turning over rapidly is missing in the case of L-ethionine substitution (Fig. 6, bottom).

In the proline-deficient host, the ^{3}H-uridine uptake is higher with azetidine-2-carboxylic acid than with L-proline substitution; however, the RNA decays similarly in the presence of the natural amino acid and the amino acid analogue both at 7 min and at 34 min after infection (Fig. 7). Thus, ^{3}H-uridine uptake and RNA decay do not correlate with the amino acid analogue-induced extension of early protein synthesis in any consistent way.

Shutoff of early protein synthesis after rifampin inhibition of RNA synthesis in the presence of amino acid analogues. Evidence presented above suggests that an active protein

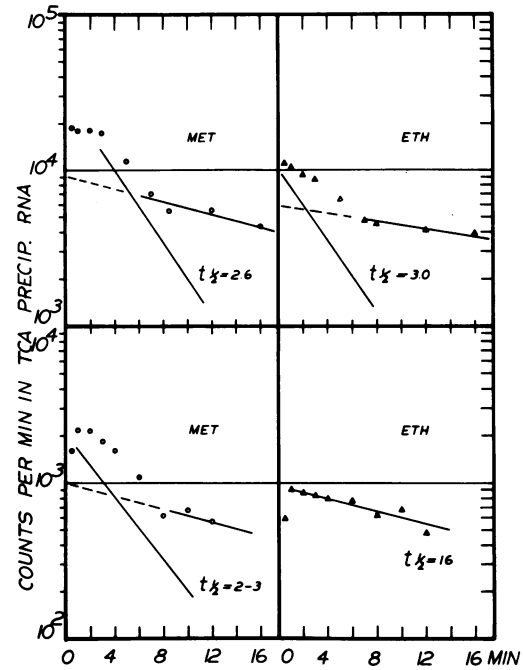

FIG. 6. *Loss of acid-precipitable RNA, pulse labeled with ^{3}H-uridine at 7 min and at 34 min after infection of E. coli B_{s-1} met⁻ with T4 43,44. Ordinate, Trichloroacetic acid-precipitable counts per minute; abscissa, time after addition of rifampin at 37 C. Experimental scheme: E. coli B_{s-1} met⁻ was infected with T4 43,44 30 min after the onset of starvation for methionine.* L-*methionine or* L-*ethionine at 10 μg/ml were added together with the phage, and the cultures were incubated at 37 C. ^{3}H-uridine was added to 5 μCi/ml at 6 min after infection. By 7 min, 200 μg of rifampin per ml was added, and the acid-insoluble, radioactive RNA was determined in 0.1-ml samples after hot SDS (1%) lysis of infective centers. Top, ^{3}H-uridine added at 6 min; bottom, ^{3}H-uridine added at 33 min after infection, rifampin at 34 min and samples taken thereafter.*

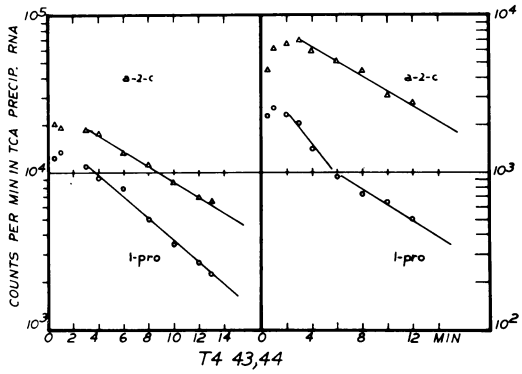

FIG. 7. *Loss of acid-precipitable RNA, pulse labeled with ^{3}H-uridine at 7 min and at 34 min after infection of E. coli B_{s-1} pro$^-$ with T4 43,44. Experimental conditions were the same as described for Fig. 6 except for starving for proline and substituting with azetidine-2-carboxylic acid (40 μg/ml). Left, ^{3}H-uridine added at 6 min after infection; right, ^{3}H-uridine added at 33 min.*

has to be synthesized after T4 infection to mediate the shutoff of synthesis of early T4 proteins. This hypothetical protein could act in four ways: (i) on the level of mRNA synthesis by closing down transcription of certain genes, (ii) by affecting the stability of mRNA, (iii) by modifying existing mRNA to render it nontranslatable, or (iv) by modifying the translational components to prevent translation of select classes of mRNA.

If the hypothetical shutoff protein were to act exclusively on the level of synthesis of mRNA (case 1), then amino acid analogues should not cause extended synthesis of early T4 proteins after RNA synthesis has been inhibited and translation has to occur from preexisting mRNA. We, therefore, infected *E. coli* B_{s-1} *met*$^-$ and B_{s-1} *pro*$^-$ with T4 DO after 30 min of starvation for the respective amino acid. To B_{s-1} *met*$^-$ were added L-methionine or L-ethionine immediately before phage addition. RNA synthesis was inhibited by rifampin addition after 10 min of incubation at 37 C of the infected cultures, and the shutoff of protein synthesis was followed by ^{14}C-amino acid pulses thereafter. The same experiment was performed with B_{s-1} *pro*$^-$. In both cases it is seen (Fig. 8 and 9) that the amino acid analogues extended the synthesis of early proteins considerably beyond the time when synthesis decreases with the natural amino acids. Provided that the amounts and compositions of T4 mRNA at the time of rifampin addition are equal in the cultures with natural amino acids and with amino acid analogues and provided that only preexisting mRNA (RNA produced before and by read-out after addition of rifampin) can be utilized, it is demonstrated that the amino acid analogues inhibit a shutoff mechanism which acts on the posttranscriptional level.

These experiments do not yet prove, however, that a protein is required to shut off early T4 genes. It is possible that the incorporation of amino acid analogues into the growing polypeptide chain in itself causes extended synthesis. To rule out this possibility, we modified the above experiments to provide for the synthesis of active T4 proteins for 10 min after infection by T4 DO, then we inhibited further RNA synthesis by rifampin addition and thereafter followed the shutoff of early protein synthesis with L-proline and with azetidine-2-carboxylic acid present. Figure 10 shows that, in spite of the presence of azetidine-2-carboxylic acid, the shutoff of synthesis of early proteins proceeds as in the case of L-proline (Fig. 9, left). It is shown, therefore, that it is indeed a protein, synthesized early after T4 infection, which is responsi-

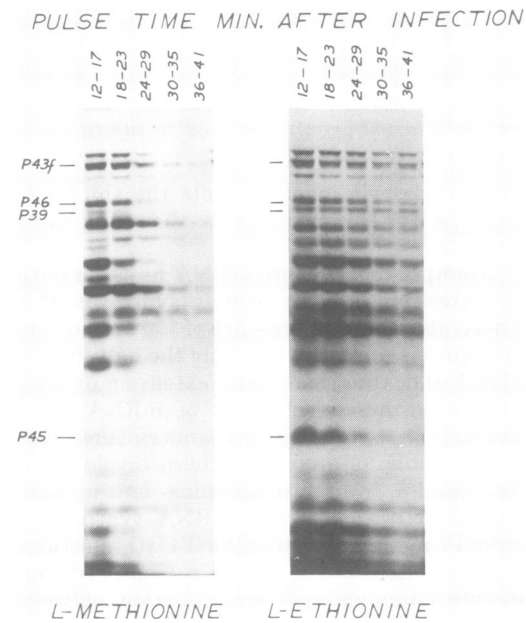

FIG. 8. *Patterns of synthesis of early T4 proteins at various times after addition of rifampin to T4 43,44-infected B_{s-1} met$^-$. Left, In the presence of L-methionine; right, in the presence of L-ethionine. Experimental scheme: B_{s-1} met$^-$, starved for methionine for 30 min, was infected with T4 43,44. To half of the culture 10 μg of L-methionine per ml was added, to the other half 10 μg of L-ethionine per ml was added together with the phage. After 10 min of incubation at 37 C, 200 μg of rifampin per ml was added and 1.0-ml samples of the cultures were pulse labeled with 2 μCi of ^{14}C-amino acids for the time indicated on the radioautographs.*

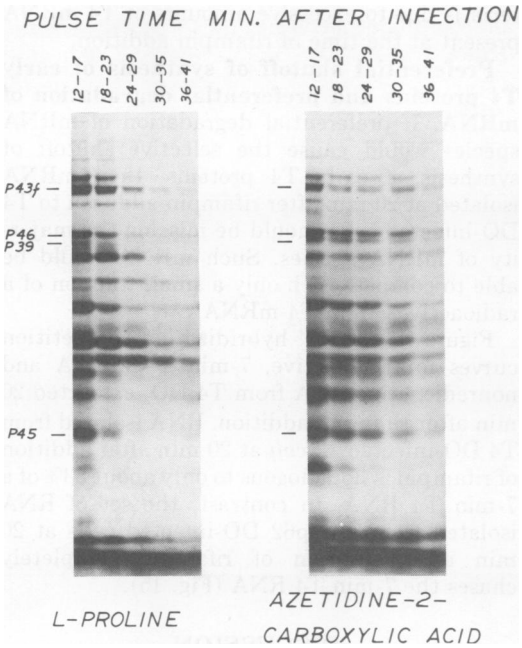

FIG. 9. *Patterns of synthesis of early T4 proteins at various times after addition of rifampin to T4 43,44-infected B_{s-1} pro⁻. Same experimental conditions as in Fig. 8 except for proline starvation and the replacement of* L-*proline by azetidine-2-carboxylic acid (40 μg/ml).*

ble for the shutoff of expression of early T4 genes in the characteristic sequence seen in Fig. 2 and 3.

Shutoff of early protein synthesis in T4 sp62 DO. Wiberg *et al.* (Fed. Proc. **30**:1263, 1971) have isolated a mutant of T4, designated sp62, which in T4 DO prevents the shutoff of synthesis of several early T4 proteins (J. S. Wiberg, S. Mendelsohn, V. Warner, K. Hercules, C. Aldrich, and J. L. Munro, in press). Recently it was (Wiberg et al., in press) observed that this mutant fails to shut off all but one or two of the early T4 proteins that can be resolved on a 10% SDS acrylamide gel (Fig. 11 left). In T4 sp62 this mutation leads to some extension of synthesis of early T4 proteins into the period of synthesis of late proteins (Fig. 11, right).

To correlate the inhibition of shutoff of early enzyme synthesis by amino acid analogues and the inhibition of sp62, we first investigated whether the failure to shut off synthesis of early T4 proteins occurs on the posttranscriptional level as well. We therefore infected *E. coli* B_{s-1} with T4 sp62 DO, inhibited RNA synthesis at 10 min after infection by addition of rifampin, and followed the pattern of protein synthesis thereafter. Figure 12 (left) shows that, in T4 sp62 DO-infected cells, there is no selective shutoff of synthesis of early T4 proteins; with T4 DO, shutoff occurs as normal (Fig. 12, right). Simultaneously we investigated the mRNA half-lives in the T4 sp62 DO- and in the T4 DO-infected cultures and observed the results shown in Fig. 13. The ^{3}H-uridine uptake is about twice as high with T4 DO, and the final rates of breakdown are similar. The absolute amounts of radioactivity in the slow turnover fractions of RNA are about equal. Thus, the observations on mRNA decay do not readily explain the selective shutoff of synthesis of certain early T4 proteins. It is

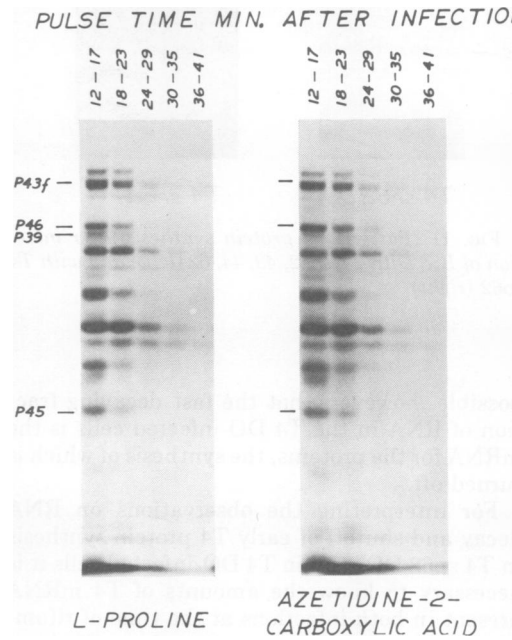

FIG. 10. *Patterns of synthesis of early T4 proteins after rifampin inhibition of RNA synthesis in T4 43,44-infected B_{s-1} pro⁻. (In this experiment synthesis of active protein was allowed for 10 min after T4 DO infection.) Left, In the presence of* L-*proline added at 10 min after infection together with rifampin; right, in the presence of azetidine-2-carboxylic acid added with rifampin at 10 min. Experimental scheme: B_{s-1} pro⁻ was proline starved for 30 min and infected with T4 43,44 incubated for 10 min at 37 C with 10 μg of* L-*proline per ml. Then rifampin was added to 200 μg/ml, the infective centers were chilled, and* L-*proline was washed out by two sedimentations, and the pellet was resuspended in M-9 medium containing 200 μg of rifampin per ml. To half of the culture was added* L-*proline (40 μg/ml), to the other half azetidine-2-carboxylic acid (40 μg/ml). ^{14}C-amino acid pulses were administered thereafter as indicated on the autoradiographs.*

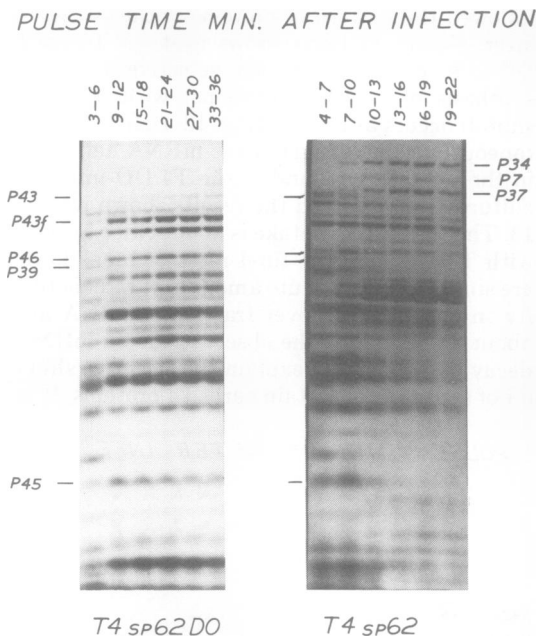

FIG. 11. *Patterns of protein synthesis after infection of B_{s-1} with T4 sp62, 43, 44, 62 (left), and with T4 sp62 (right).*

possible, however, that the fast decaying fraction of RNA in the T4 DO-infected cells is the mRNA for the proteins, the synthesis of which is turned off.

For interpreting the observations on RNA decay and shutoff of early T4 protein synthesis in T4 sp62 DO- and in T4 DO-infected cells it is necessary to know the amounts of T4 mRNA present in both infections at the time of rifampin addition (10 min after infection). We have therefore extracted nonradioactive RNA from T4 sp62 DO- and from T4 DO-infected cells at 10 min after infection and assayed for the relative amounts of T4-specific RNA in both preparations. Two separate RNA extractions were performed from T4 sp62 DO- and from T4 DO-infected cells. Equal amounts of RNA were recovered from the four cultures. The RNAs were then used to compete against radioactive RNA extracted at 7 min after infection by T4 wild type. The results are shown in Fig. 14. It is seen that, in the T4 sp62 DO-infected cultures, only half as much T4 RNA was present as in the T4 DO-infected cultures. This observation agrees with the relative rates of ^{3}H-uridine uptake observed with T4 sp62 DO and T4 DO (see Fig. 13). It is therefore ruled out that the extended synthesis of early T4 proteins after rifampin addition to T4 sp62 DO-infected bacteria is due to excessive amounts of T4 mRNA present at the time of rifampin addition.

Preferential shutoff of synthesis of early T4 proteins and preferential degradation of mRNA. If preferential degradation of mRNA species would cause the selective shutoff of synthesis of early T4 proteins, then mRNA isolated at 20 min after rifampin addition to T4 DO-infected cells should be missing the majority of mRNA species. Such mRNA would be able to compete with only a small fraction of a radioactive 7-min T4 mRNA.

Figure 15 shows hybridization-competition curves for radioactive, 7-min T4 mRNA and nonradioactive RNA from T4 DO, extracted 20 min after rifampin addition. RNA isolated from T4 DO-infected *E. coli* at 20 min after addition of rifampin is homologous to only about 33% of a 7-min T4 RNA. In contrast, the set of RNA isolated from T4 sp62 DO-infected cells at 20 min after addition of rifampin completely chases the 7-min T4 RNA (Fig. 15).

DISCUSSION

In T4 DO-infected cells, synthesis of early T4 proteins is terminated in the characteristic, sequential pattern displayed in Fig. 2. The shutoff pattern seen with T4 DO is similar to the one observed with T4 wild type, which

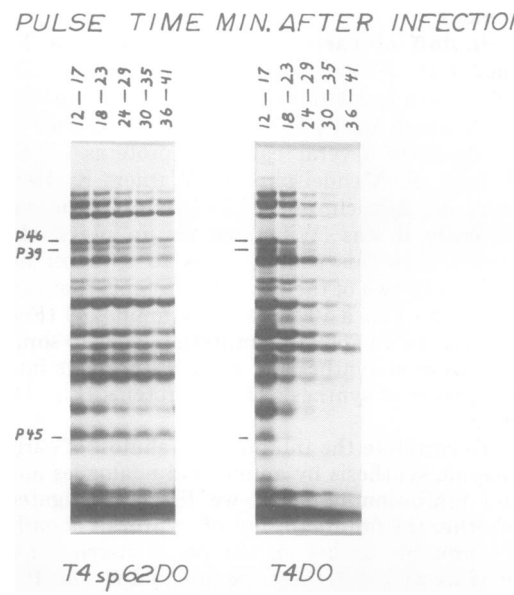

FIG. 12. *Patterns of protein synthesis in T4 sp62, 43,44,62- and T4 43,44,62-infected B_{s-1} after rifampin addition at 10 min after infection. Left, T4 sp62 DO; right, T4 DO.*

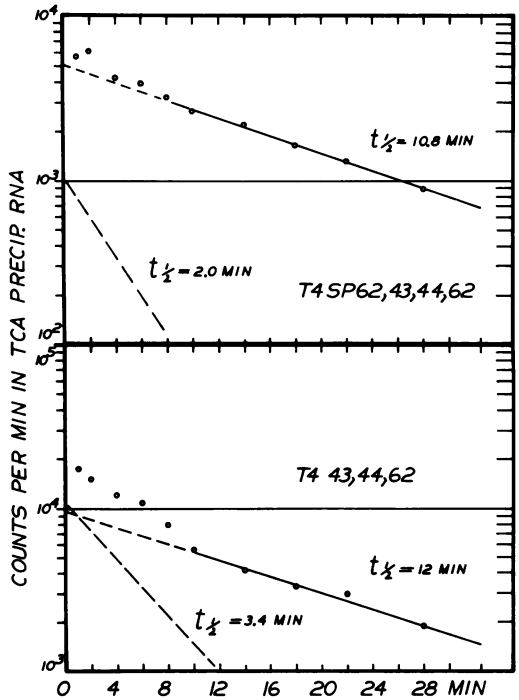

FIG. 13. *Loss of acid-precipitable RNA, pulse labeled with ^{3}H-uridine at 10 min after infection of B_{s-1} with T4 sp62 43,44,62 and with T4 43,44,62. Ordinate, trichloroacetic acid-precipitable counts per minute; abscissa, time after addition of 200 μg of rifampin per ml. Rifampin was added 1 min after addition of ^{3}H-uridine.*

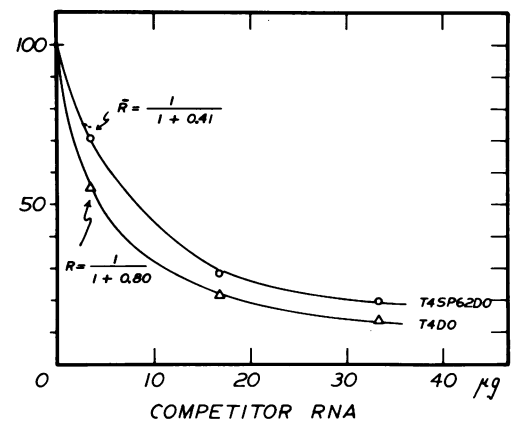

FIG. 14. *DNA-RNA hybridization-competition curves obtained with radioactive early T4 RNA, isolated 7 min after infection with T4 wild type and nonradioactive competitor RNA from B_{s-1} infected with either T4 sp62, 43, 44, 62 or T4 43, 44, 62 and isolated 10 min after infection at 37 C. Ordinate, Normalized percent of radioactive RNA hybridized with DNA; abscissa, amount of competitor RNA added. $R = 1/(1 + C)$ is the ratio of radioactive RNA over the sum of the radioactive plus nonradioactive competitor RNA. Input radioactive RNA, 2 μg.*

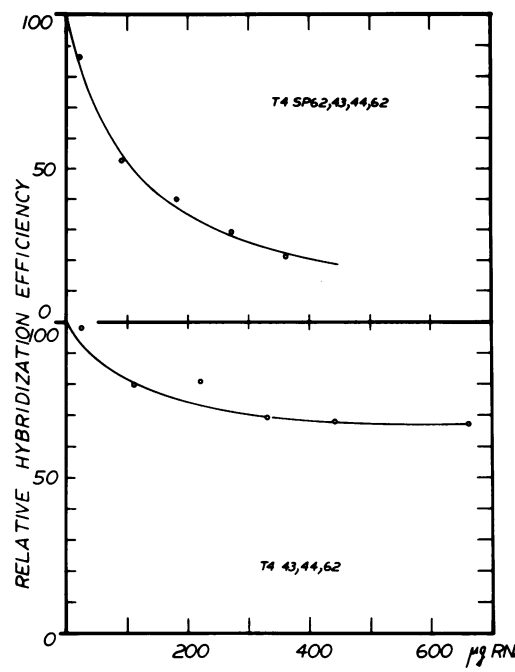

FIG. 15. *DNA-RNA hybridization-competition curves obtained with the same radioactive, early T4 RNA as in Fig. 14, and competitor T4 RNA isolated from B_{s-1} at 20 min after inhibition of RNA synthesis by 200 μg of rifampin per ml. Bottom, Nonradioactive competitor RNA from T4 43,44,62-infected B_{s-1}; top, from T4 sp62, 43, 44, 62-infected cells. In both cultures rifampin was added at 10 min after infection by T4 phages.*

suggest that the same mechanisms are active in both. The continuation of synthesis of a fraction of the early T4 proteins after 20 min of infection by T4 DO cannot be fully accounted for by the preferential synthesis of mRNA, since RNA synthesized late after T4 DO infection is largely (although not completely) homologous to RNA synthesized early after infection (unpublished results). It seems likely, therefore, that the shutoff of synthesis of select early T4 proteins acts on the posttranscriptional level (as suggested earlier by Khesin et al. [8] and Hall et al. [4]).

When the T4 DO infection is carried out in the presence of amino acid analogues, the selective shutoff of synthesis of early T4 proteins is no longer observed. It thus appears that the shutoff depends upon the synthesis of active proteins after T4 infection. We assume the shutoff function to be under the control of a T4 gene.

This contention is verified by the experiments in which RNA synthesis is inhibited by rifampin and the shutoff of early protein synthesis is

monitored in the presence and the absence of amino acid analogues (Fig. 8 and 9). The fact that shutoff is prevented by the presence of the amino acid analogues points to the requirement of an active T4 protein. It was necessary, however, to verify that it is not the presence per se of the amino acid analogues but their inhibiting the synthesis of active proteins which prevents the shutoff of early protein synthesis. In experiments in which the T4 DO infection is carried out in the presence of natural amino acids to allow for the synthesis of active T4 proteins, which is followed by rifampin inhibition of RNA synthesis and then by analogue substitution of amino acids, synthesis of early T4 proteins is shut off as usual. We may therefore conclude that the shutoff of early T4 protein synthesis acts after the mRNA has been synthesized.

One of us (K. H.) recently observed that sp62 prevents the shutoff of synthesis of most early T4 proteins in T4 DO-infected cells. We have followed T4 protein synthesis in sp62 DO after rifampin inhibition of RNA synthesis and we now observe that the loss of shutoff occurs on the posttranscriptional level. Since the same result is obtained with T4 DO phage in the presence of amino acid analogues, it becomes likely that sp62 controls a protein that affects the shutoff of synthesis of early T4 proteins.

Since the shutoff mechanism acts after the message has been synthesized, it could do so by modifying the message to render it nontranslatable or by altering the components of the machinery of protein synthesis to bring about preferential or exclusive utilization of certain mRNA species. An extreme case of message modification would be selective degradation. From the mRNA turnover studies in the presence and absence of amino acid analogues (Fig. 6 and 7) we cannot draw any conclusion concerning selective degradation. With starvations for different amino acids and with substituting different amino acid analogues, we obtain results on mRNA half-lives which vary in a fashion inconsistent with any simple interpretation. However, comparing mRNA decay in T4 sp62 DO- and T4 DO-infected cells, we observe a slow decay of mRNA affecting almost all species in the case of T4 sp62 DO and different rates of RNA turnover discriminating between at least two sets of RNA in the case of T4 DO. About 70% of the RNA of T4 DO displays a fast turnover ($t_{1/2}$ = 2–4 min); the remainder displays the same, slow decay as the majority of the RNA in T4 sp62 DO-infected cells ($t_{1/2}$ = 10–12 min).

We observe that the fast decaying fraction of T4 DO RNA is a select class of early T4 mRNA, because the RNA persisting until 20 min after rifampin addition is homologous to only a small fraction of the early T4 RNA. In contrast, RNA persisting 20 min after rifampin addition to T4 sp62 DO-infected cells is completely homologous to early T4 mRNA (Fig. 15).

We have seen that the selective shutoff of synthesis of early T4 proteins in T4 DO-infected cells is accompanied by a selective breakdown of mRNA. However, we do not know whether the selective breakdown of T4 mRNA is the cause or the consequence of the selective termination of synthesis of early T4 proteins.

We have performed the following experiments which may be pertinent to this question. When the overall protein synthesis was inhibited by puromycin (10) in T4 DO- and in T4 sp62 DO-infected cells we did not observe an accelerated breakdown of T4 mRNA. Therefore, lack of translation of mRNA due to premature termination of protein chains does not lead to accelerated degradation of T4 mRNA. This observation may indicate that the selective breakdown of T4 mRNA is the cause of the selective shutoff of synthesis of early T4 proteins in infections with DNA-negative mutants of T4.

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