F-Factor-Mediated Restriction of Bacteriophage T7: Synthesis of RNA and Protein in T7-Infected *Escherichia coli* F− and F+ Cells

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Bacteriophage T7 is unable to productively infect *Escherichia coli* strains carrying the sex factor F. T7 phage development, in terms of RNA and protein synthesis, was compared in T7-infected isogenic F− and F+ strains of *E. coli*. Slightly less T7 early mRNA and early protein were synthesized in F+ cells. In addition to the defect in T7 late protein production in F+ cells reported by others, significantly less T7 late mRNA was synthesized, about one-half of that produced in T7-infected F− cells. Moreover, host RNA synthesis was not completely inhibited. The protein-synthesizing ability of T7-infected F+ cells decayed much faster than that of F− cells both in vivo and in vitro. This faster decay appears to explain the failure of F+ cells to produce T7 late protein in vivo, even in the presence of a considerable amount of translatable T7 late mRNA. Therefore, it may not be necessary to postulate the involvement of specific translational discrimination against T7 late mRNA, although it appears that F-factor-mediated restriction of T7 involves changes in transcription as well as translation.

Infection of *Escherichia coli* strains carrying the sex factor F with bacteriophage T7 results in an immediate cessation of growth and eventual death of the infected cells. However, T7 infection ends abortively; normal liberation of progeny phages does not occur. This phenomenon of F-factor-mediated restriction of T7 development has been explained by a specific inhibition of the host-translational system by F-factor-coded product(s), thereby blocking the synthesis of phage late proteins directed by T7 late mRNA. Morrison and Malamy (12) found little or no late proteins in T7-infected F+ cells despite the presence of T7 late mRNA. Recent work by Blumberg and Malamy (1) showed the presence of a considerable amount of untranslated T7 late mRNA which, after extraction from the T7-infected cells, can be translated in vitro in a cell-free protein-synthesizing system. Furthermore, certain mutations in the F factor permit the F+ cells to synthesize T7 late proteins and complete a productive lytic cycle (12). These results suggest that translational control is involved in the switch from the synthesis of T7 early proteins to late proteins in the natural host F− cells, and that F-factor-coded product(s) specifically inhibits this T7 late mRNA translation system in F+ cells.

However, no evidence of translational discrimination between T7 early mRNA and late mRNA was found in T7-infected F− cells. Our previous experiments demonstrated that cell-free protein-synthesizing systems prepared from T7-infected F− cells show no difference in their ability to translate T7 early mRNA or late mRNA and produce respective proteins (15). This lack of expected specificity for T7 mRNA species was found in both T7-infected and uninfected cells.

In addition, we have provided evidence that T7 early mRNA is functionally unstable, although the RNA is stable in chemical terms and remains in the cell late in T7 infection (16, 17). This functional decay of T7 early mRNA appears to be sufficient to explain the preferential synthesis of T7 late proteins late in T7 infection in the presence of both early mRNA and late mRNA without translationally discriminating early mRNA in the natural T7 host F− cells.

The apparent lack of specific translational control in T7-infected F− cells, described above, prompted us to examine factors which would explain the phenomenon of F-factor-mediated restriction of T7 development. In this paper we present dissimilarities between F− and F+ cells in the process of T7 development. Using DNA-RNA hybridization to quantitatively measure T7-specific RNA, we found a slight but significant reduction in the amount of T7 early mRNA and early protein and a large reduction in the amount of T7 late mRNA and late protein in T7-infected F+ cells compared to that in F− cells. In addition, T7-infected F+ cells failed to
exert a complete "host shut-off" function of T7 and resulted in a prolonged residual synthesis of host RNA.

The protein-synthesizing activity of T7-infected F+ cells decayed much faster than that of F- cells both in vivo and in vitro. This difference explains the failure of F+ cells to produce T7 late proteins in vivo in the presence of a considerable amount of translatable T7 late mRNA without necessitating a specific translational discrimination against T7 late mRNA.

It appears that F-factor-mediated restriction of T7 involves factors affecting transcription as well as translation.

**MATERIALS AND METHODS**

**Bacteria and T7 phages.** Our stock of *E. coli* D10 is an F+ strain and requires methionine and thiamine. D10 F- was isolated from D10 F+ by treatment with acridine orange following the method of Hirota (10). Wild-type and mutant T7 phages were from the collection of F. W. Studier. T7 am27 contains an amber mutation in the gene 1 coding for T7-specific RNA polymerase, and T7 13a is an amber mutant (gene 3.5) which fails to produce active T7 lysozyme.

T7 infection. D10 F+ and F- cells were grown in an M9-glucose medium supplemented with 40 μg of l-methionine per ml and 25 μg of thiamine per ml at 30°C with shaking. Doubling time of both F+ and F- strains was about 45 min. At a cell density of 5 × 10^6/ml, the cultures were infected with T7 phage at a multiplicity of infection of 5.

**RNA and protein synthesis in T7-infected cells.** The synthesis of total RNA and protein in T7-infected cells was followed by measuring the incorporation of [1^4]C]uracil (0.1 μCi/2 μg per ml) into cold trichloroacetic acid-insoluble material and [3H]phenylalanine (2 μCi/3 μg per ml) into hot trichloroacetic acid-insoluble material, respectively. For the RNA/binding labeling of T7-specific RNA to be used for the hybridization experiments, [3H]uridine (10 μCi/5 μg per ml) was added to the culture 10 min before T7 infection to maintain a constant specific activity of the RNA precursor pool during the period of phage RNA synthesis.

**Rate of RNA and protein synthesis in T7-infected cells.** Rate of RNA synthesis was measured by the incorporation of [3H]uridine into cold trichloroacetic acid-insoluble material in 1-min labeling time at 30°C. Aliquots of 25 ml were removed from T7-infected culture at various times and the incubation was continued for an additional 1 min in the presence of [3H]uridine (2 μCi/1 μg per ml). Rate of protein synthesis was measured by the incorporation of [3H]phenylalanine into hot trichloroacetic acid-insoluble material in 1 min at 30°C. At various times after T7 infection, 0.2-ml aliquots of the culture were transferred to 0.2 ml of prewarmed media containing [3H]phenylalanine (final concentration: 2 μCi/0.1 μg per ml) and the incubation was continued for an additional 1 min.

**Preparation of RNA from T7-infected cells for hybridization.** RNA was extracted from T7-infected cells by the method of Summers (14), which included lysozyme treatment of the cells and lysis of the resulting protoplasts with a detergent. RNA was extracted from the lysate with phenol four times, precipitated with ethanol, and suspended in 4 x 0.15 M NaCl, 0.015 M sodium citrate containing 0.1% sodium dodecyl sulfate. The concentration of RNA was estimated by the absorbancy (260 nm) value.

**DNA-RNA hybridization.** Hybridization of radioactive RNA from T7-infected cells with T7 DNA was carried out following the method of Cooper et al. (7). T7 DNA isolated from purified T7 phage by phenol extraction, about 1 mg, was bound to membrane filter (90-mm diameter, Millipore Corp.), and about 100 small disks (6-mm diameter) were cut out. Each hybridization reaction used one 6-mm DNA filter disk and 50 μl of 4 x 0.15 M sodium dodecyl sulfate and radioactive RNA sample. After an incubation at 66°C for 16 h, filter disks were washed with 2 x 0.15 M SSC and treated with RNase, and the amount of radioactivity bound to the filter was counted. Radioactive RNA from uninfected cells was also used to hybridize with T7 DNA to measure the background count. Usually the background count was less than 0.01% of total radioactivity of the RNA added to the hybridization reaction. In all hybridization experiments, the amount of mRNA was kept in excess of T7-specific RNA by about 10-fold.

**T7-specific RNA polymerase assay.** At various times after T7 infection, 25-ml aliquots of the culture were poured over crushed ice and the cells were pelleted by centrifugation. The cells were suspended in 1 ml 0.01 M Tris-hydrochloride, pH 7.9, 0.01 M MgCl_2 containing 0.01 M mercaptoethanol and then disrupted by sonication (Heat Systems-Ultrasonics Inc., model W185D) for 2 min. After removing cell debris by a 10-min centrifugation at 12,000 × g, 0.02 ml of the cell-free extract was assayed for T7-specific RNA polymerase activity by the procedure described by Chamberlin et al. (4). In each reaction, 10 μCi of [3H]UTP (20 Ci/mmol), and 4 μg of T7 DNA. The reaction mixture was incubated at 37°C for 10 min and cold trichloroacetic acid-insoluble radioactivity was counted. The activity was expressed as counts per minute of [3H]UMP incorporated per milligram of cell-free extract protein. Since rifampin inhibits only *E. coli* RNA polymerase (4), this assay is specific for T7-specific RNA polymerase.

**E. coli RNA polymerase assay.** T7-infected cells were harvested at various times after infection, suspended in 0.05 M Tris-hydrochloride, pH 7.6, containing 0.01 M MgCl_2, 0.2 M KCl, 0.1 mM dithiothreitol, and 0.1 mM EDTA, and sonically treated as described above. Following the method of Burgess (3), sonically treated cell-free extract was treated with DNase to remove endogenous DNA and then fractionated by ammonium sulfate precipitation. The 35 to 50% ammonium sulfate fraction was dissolved in 0.1 M Tris-hydrochloride, pH 7.9, 0.01 M MgCl_2 containing 0.01 M mercaptoethanol and then disrupted by sonication (Heat Systems-Ultrasonics Inc., model W185D) for 2 min. After removing cell debris by a 10-min centrifugation at 12,000 × g, 0.02 ml of the cell-free extract was assayed for T7-specific RNA polymerase activity by the procedure described by Chamberlin et al. (4). In each reaction, 10 μCi of [3H]UTP (20 Ci/mmol), and 4 μg of T7 DNA. The reaction mixture was incubated at 37°C for 10 min and cold trichloroacetic acid-insoluble radioactivity was counted. The activity was expressed as counts per minute of [3H]UMP incorporated per milligram of cell-free extract protein. Since rifampin inhibits only *E. coli* RNA polymerase (4), this assay is specific for T7-specific RNA polymerase.
coli RNA polymerase enzyme was assayed. The amount of material was completely precipitated with MgCl₂, 0.1 mM mercaptoethanol, 50 mM each of ATP, CTP, GTP, and UTP, 1 μCi of [³H]UTP, and 4 μg of T₄ DNA. The assay mixture was incubated at 37°C for 10 min and the radioactivity in trichloroacetic acid-insoluble material was counted. The activity of the enzyme was expressed as counts per minute of [³H]UMP incorporated per milligram of protein. This assay was confirmed to be specific for E. coli RNA polymerase because the enzyme activity depended entirely on the added T₄ DNA template and rifampin completely inhibited the enzyme activity.

T₇ lysozyme assay: Sonically treated cell-free extracts were prepared from T₇-infected cells as described above. A 0.04-ml amount of cell-free extract was added to 0.96 ml of 0.1 M ammonium acetate and assayed for T₇ lysozyme activity as described by Hagen and Young (8). The assay measures the solubilization of [³H]diaminopimelic acid from filter disks on which [³H]diaminopimelic acid-labeled E. coli cell walls had been precipitated. The activity was expressed as counts per minute of [³H]diaminopimelic acid released per milligram of cell-free extract protein.

T₇ lysozyme mRNA assay. RNA was extracted from T₇-infected cells at various times after infection and assayed for T₇ lysozyme mRNA activity in an in vitro protein-synthesizing system prepared from uninfected F⁻ cells. Incubated cell-free extract (IS₉₀) was prepared as described previously (15). Twenty-five microliters of RNA was added to 0.05 ml of in vitro protein-synthesizing system and incubated at 37°C for 45 min. T₇ lysozyme produced was assayed by measuring the activity of T₇ lysozyme in 0.02 ml of the reaction mixture, following the procedure described previously (15).

Protein-synthesizing activity of cell-free systems from T₇ 13a-infected cells. F⁻ and F⁺ cells were infected with T₇ 13a (an amber mutant in gene 3.5 coding for T₇ lysozyme) to eliminate a high level of endogenous T₇ lysozyme activity. IS₉₀ were prepared from the cells harvested at various times after T₇ infection. RNA extracted from F⁻ cells harvested 10 min after wild-type T₇ infection was used as the messenger to direct T₇ lysozyme synthesis in vitro. The reaction mixture, in a total volume of 0.05 ml, contained: 25 μg of RNA from T₇-infected cells as messenger, 300 μg (protein content) of IS₉₀, 54 mM Tris-hydrochloride, pH 7.9, 7 mM MgCl₂, 50 mM potassium acetate, 59 mM NH₄Cl, 2.5 mM dithiothreitol, 2 mM ATP, 0.5 mM GTP, 0.1 mM each of 20 amino acids, 25 μg of stripped E. coli tRNA, 20 mM phosphenyl pyruvate, 2 mM spermidine, 2.5 mM polyethylene glycol. After an incubation at 37°C for 45 min, 0.02 ml of the reaction mixture was assayed for the activity of synthesized T₇ lysozyme.

Determination of protein. Protein content of all samples described in this paper was determined by the method of Lowry et al. (11).

RESULTS

Lysis of T₇-infected F⁻ and F⁺ cells. The result shown in Fig. 1 compares the time course of cell lysis in T₇-infected isogenic F⁻ and F⁺ strains of E. coli. In both strains, T₇ infection resulted in an immediate inhibition of cell growth measured by the turbidity of the culture, and the number of colony-forming units of the culture decreased by at least 100-fold. At 25 to 30 min after infection, the F⁺ culture underwent a rapid lysis yielding about 150 to 200 progeny phages per cell. In contrast, the F⁻ culture showed only a slight decrease in turbidity and no significant increase in the number of infectious progeny phages.

Synthesis of RNA and protein in T₇-infected F⁻ and F⁺ cells. Measurement of total RNA and protein synthesis in T₇-infected F⁻ and F⁺ cells revealed distinct differences in both RNA and protein synthesis. Figure 2 shows the incorporation of radioactive precursors into RNA (Fig. 2A) and protein (Fig. 2B) throughout T₇ infection in F⁻ and F⁺ cells as compared with that in uninfected cells. The results in Fig. 2 clearly shows that less RNA and protein were synthesized in the F⁺ cells compared to the F⁻ cells. The result also indicates that the rate of RNA and protein synthesis in T₇-infected F⁺ cells decreased much earlier than that in F⁻ cells.

The rate of synthesis of RNA and protein in T₇-infected F⁻ and F⁺ cells was measured by pulse labeling the infected cells for 1 min at different times after infection with radioactive uridine and phenylalanine, respectively. The result shown in Fig. 3 confirmed the notion

![Fig. 1. Time course of cell lysis in T₇-infected F⁻ and F⁺ cells. F⁻ and F⁺ cells were grown in M9-glucose medium at 30°C and infected with T₇ phage at a multiplicity of infection of 5. Turbidity of the infected cultures was measured by a Klett-Summerson colorimeter with 530 filter.](http://jvi.asm.org/)

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Fig. 2. Synthesis of RNA (A) and protein (B) in T7-infected F− and F+ cells. (A) Synthesis of RNA in T7-infected cells. At the time of T7 infection, [14C]uracil (0.1 μCi/2 μg per ml) was added to the culture. At indicated times 0.2 ml of the culture was removed and cold trichloroacetic acid-insoluble radioactivity was counted. (B) Synthesis of protein in T7-infected cells. At the time of T7 infection, [3H]phenylalanine (2 μCi) was added to the culture. At indicated times 0.2 ml of the culture was removed and hot trichloroacetic acid-insoluble radioactivity was counted. Dotted lines show the incorporation of radioactive materials in uninfected F− cells.

Fig. 3. Rate of RNA and protein synthesis in T7-infected F− and F+ cells. (A) Rate of RNA synthesis in T7-infected cells. At indicated times after infection, aliquots of the culture (25 ml) were removed and pulse-labeled with [3H]uridine (2 μCi/μg per ml) for 1 min at 30 C. RNA was extracted from each pulse-labeled sample, and RNA solution (1 mg/ml) was prepared. Radioactivity in cold trichloroacetic acid-insoluble material in 20 μl of the RNA solution was counted. The results were expressed as percentage of the zero time value (F−, 45,618 counts/min; F+, 44,241 counts/min). (B) Rate of protein synthesis in T7-infected cells. At indicated times after infection, 0.2 ml of the culture was removed and pulse labeled with [3H]phenylalanine (2 μCi/0.1 μg per ml) for 1 min at 30 C and the hot trichloroacetic acid-insoluble radioactivity was counted. The results were expressed as percentage of the zero time value (F−, 9,765 counts/min; F+, 9,150 counts/min).
indicated from the result in Fig. 2 that the rate of synthesis of RNA and protein decreased much earlier in F+ cells than F− cells. Special attention was given to a faster decline of the rate of protein synthesis in F+ cells compared to that in F− cells as will be discussed later in this paper.

Synthesis of T7-specific RNA in T7-infected F− and F+ cells. In both F− and F+ cells infected with T7, a residual synthesis of host RNA and protein continues for several minutes after infection until the action of the "host shut-off" function of T7, which inactivates the host RNA polymerase, becomes complete (9, 13). Therefore, total RNA and protein synthesized after T7 infection includes some amounts of host RNA and protein. We used DNA-RNA hybridization to determine the amount of T7-specific RNA synthesized in T7-infected F− and F+ cells. Radioactive uridine-labeled RNA synthesized in T7-infected cells was hybridized with T7 DNA to measure the amount of T7-specific RNA including T7 early mRNA and late mRNA. As shown in Fig. 4, total amount of T7-specific RNA produced in T7-infected F− cells was remarkably less that that in F+ cells.

The reduced amount of RNA synthesized in T7-infected F− was shown to be due to a decrease in the rate of T7-specific RNA synthesis, particularly late in infection, in F+ cells as illustrated in Fig. 5. The result shown in Fig. 5 was obtained by 1 min-pulse labeling of T7-infected cells with radioactive uridine at different times after infection and measuring the radioactivity of T7 DNA-hybridized RNA. The results in Fig. 4 and 5 suggest that in T7-infected F+ cells a comparable amount of T7 early mRNA was produced relative to that in F− cells but the amount of T7 late mRNA produced in F− cells was greatly reduced, to only about one-half of the amount in F+ cells, because T7 early mRNA synthesis ceases at about 8 min (see Fig. 6).

An attempt was made to quantitatively measure the proportion of T7 early mRNA and late mRNA contained in the total T7-specific RNA by a two-step competitive hybridization. Filters with bound T7 DNA were incubated with or without a saturating amount of nonradioactive T7 early mRNA prepared from F− cells infected with T7 in the presence of chloramphenicol (16). Then a second hybridization with radioactive RNA isolated from T7-infected cells at different times after infection was carried out. Although we obtained an indication that at least about 90% of total T7-specific RNA should be composed of T7 late mRNA late in T7 infection, the presence of such a dominating amount of T7 late mRNA among the total T7-specific RNA in the RNA samples from late T7 infection period made an accurate measurement of T7 early mRNA and late mRNA very difficult (data not shown).

However, this information that total T7-specific RNA synthesized in both F− and F+ cells was dominated by T7 late mRNA further supports the notion that a greatly reduced amount of T7-specific RNA produced in F+ cells is due mainly to a great reduction of T7 late mRNA synthesis as already indicated from the results shown in Fig. 4 and 5.

Synthesis of T7 early mRNA and early protein in T7-infected F− and F+ cells. Use of an amber mutant of T7 phage, T7 am27, with a mutation in gene 1, which codes for the T7-specific RNA polymerase, allowed us to compare the relative amount of T7 early mRNA in
Host shut-off function of T7 in F− and F+ cells. In normal T7 infection of the natural host F− cells, an inactivation of the host RNA polymerase transcribing the host RNA and T7 early mRNA occurs at about the same time that T7-specific RNA polymerase, an early gene product of T7, begins to transcribe T7 late mRNA from the late genes (9). The host shut-off function of T7 was shown to be an inactivation of the host RNA polymerase by an inhibitor protein (9) which is supposedly one of the T7 early proteins (13).

Simple assay for the host shut-off function can be accomplished by partially purifying the host RNA polymerase from T7-infected cells to remove endogenous DNA. The results of such an experiment, shown in Fig. 8, indicates that the decline of the host RNA polymerase activity assayed in vitro was slower and incomplete in T7-infected F+ cells in comparison to the F− cells. In F− cells the host enzyme was usually inactivated completely by the 10th min of T7

F− and F+ cells. Since the cells infected with the mutant phage do not produce T7 late mRNA due to the lack of T7-specific RNA polymerase, T7 DNA-hybridizable RNA synthesized after infection is solely T7 early mRNA. Figure 6 shows the result of such an experiment. The amount of T7 early mRNA synthesized in F+ cell was slightly but significantly less than that in F− cells. The reduction was somewhere between 20 to 25% in repeated experiments.

The slight reduction in the amount of T7 early mRNA in F+ cells was accompanied by a slight reduction in the amount of T7 early protein produced in the T7-infected F+ cells. This was shown in an experiment which compared the amount of T7-specific RNA polymerase, a T7 early protein, produced in wild-type T7-infected F− and F+ cells as illustrated in Fig. 7. T7-specific RNA polymerase synthesized in the F+ cells was about 70 to 80% of the amount of that found in T7-infected F− cells.

From these results, we conclude that in T7-infected F+ cells T7 early development proceeds almost normally except that the amount of both early mRNA and early protein produced in the F+ cells was significantly less than that in F− cells.
infection as previously reported (9).

This incomplete host shut-off observed in T7-infected F+ cells was reflected in the composition of the RNA synthesized in F+ cells after the infection with T7. From the experiments which measured the total amount of radioactive RNA (Fig. 3A) and the amount of T7 DNA-hybridizable RNA (Fig. 5), the proportion of T7-specific RNA among the total RNA synthesized in a 1-min pulse-labeling period was calculated. Ten min after T7 infection and thereafter, almost all the RNA synthesized in F- cells was hybridizable to T7 DNA, considering the efficiency of the hybridization reaction. On the other hand, only about one-half of the RNA synthesized in T7-infected F+ cells hybridized with T7 DNA (Fig. 9). We assume that the presence of a large proportion of the host RNA among the total RNA synthesized after T7 infection of F+ cells is due at least in part to the incomplete inactivation of the host RNA polymerase in addition to the large reduction in the production of T7 late mRNA which can contribute to the change of the ratio of T7-specific RNA and non-T7 RNA.

Synthesis of T7 late mRNA and late protein in T7-infected F- and F+ cells. To quantitate the difference in the amount of T7 late gene products synthesized in T7-infected F- and F+ cells, we compared the amount of T7 lysozyme mRNA and the T7 lysozyme, one of the late proteins coded by gene 3.5, produced in F- and F+ cells after T7 infection.

The amount of lysozyme mRNA was measured by assaying the activity of lysozyme synthesized in an in vitro protein-synthesizing system in the presence of RNA isolated from T7-infected cells harvested at different times after infection. T7-infected F+ cells produced translatable T7 late mRNA, represented by the lyso-

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**Fig. 7.** Synthesis of T7-specific RNA polymerase in T7-infected F- and F+ cells. At indicated times after T7 infection, aliquots of the culture were removed and sonically treated cell-free extracts were prepared as described in Materials and Methods. The activity of T7-specific RNA polymerase of each extract was assayed as described in the presence of T7 DNA and rifampin. The activity was expressed as counts per minute of [3H]UMP incorporated into RNA per milligram of protein of the extract.

**Fig. 8.** Host shut-off effect in T7-infected F- and F+ cells. At indicated times after T7 infection, aliquots of the culture were withdrawn and sonically treated cell-free extracts were prepared and fractionated with (NH4)2SO4. The 33 to 50% (NH4)2SO4 fraction of each extract was assayed for the activity of E. coli RNA polymerase using T4 DNA as the template as described. The activity of E. coli RNA polymerase was expressed as counts per minute of [3H]UMP incorporated into RNA per milligram of protein. The 100% values were: F-, 5,250 counts/min; F+, 5,488 counts/min.
Fig. 9. Efficiency of host shut-off in T7-infected F− and F+ cells. (Percentage of total radioactive RNA synthesized during 1-min pulse which is hybridizable to T7 DNA.) At indicated times after infection, aliquots of the culture were removed and pulse-labeled with [3H]uridine for 1 min and [3H]uridine-labeled RNA as isolated from each sample. A 20-μl portion of the RNA (1 mg/ml of solution) was used for the measurement of trichloroacetic acid-insoluble 3H radioactivity (Fig. 3A) and another 20-μl portion of the RNA was used for the T7 DNA-hybridizable 3H radioactivity (Fig. 5). From the results shown in Fig. 3A and Fig. 5, the percentage of T7 DNA-hybridizable radioactivity among the total trichloroacetic acid-insoluble radioactivity in 20 μl of each RNA sample was calculated.

zyyme mRNA, but only a reduced amount compared to the F− cells (Fig. 10). This is in a good agreement with the result of Blumberg and Malamy (1) except that in the present experiment lysozyme mRNA produced in F+ cells was about one-half of that in F− cells, whereas about 75% as much translatable lysozyme mRNA was found in F+ cells by Blumberg and Malamy (1).

On the other hand, the amount of T7 late protein synthesized in T7-infected cells was greatly reduced. Figure 11 shows the appearance of the activity of T7 lysozyme in T7-infected F− and F+ cells. Compared to F− cells, F+ cells produced only at most about 10% as much lysozyme in spite of the presence of about 50% as much translatable lysozyme mRNA. However, since both F− and F+ cells infected with mutant T7 phage, T7 am27, or T7 13a did not produce any detectable amount of lysozyme, the amount of lysozyme synthesized in T7-infected F+ cells was significantly higher than the background level.

It is interesting to note that in F− cells the amount of lysozyme mRNA decayed rather rapidly after the maximum value was attained 10 min after infection (Fig. 10). A similar decay of T7 lysozyme mRNA was also observed by Hagen and Young (8). On the other hand, T7 late mRNA appeared to be chemically stable, as indicated from the result shown in Fig. 4, since total T7-specific RNA is composed of mostly T7 late mRNA. Therefore, T7 late mRNA can be considered as functionally unstable but chemically stable mRNA, a similar property already found with T7 early mRNA (16, 17).

It was also noticed that the amount of T7 lysozyme in T7-infected F− cells declined rapidly (Fig. 11). This decline is perhaps due to a partial loss of the enzyme from the cells accompanying the progress of cell lysis late in T7

Fig. 10. Synthesis of T7 late mRNA in T7-infected F− and F+ cells. At indicated times after T7 infection, aliquots of the culture were removed and RNA was isolated from each sample. The RNA was added to an in vitro protein-synthesizing system prepared from uninfected F− cells and T7 lysozyme synthesized in vitro was assayed for its activity as described. The activity of lysozyme synthesized in vitro was plotted as the measure of T7 late mRNA (lysozyme mRNA) content in each sample.
infection as has been observed by Hagen and Young (8).

In vitro synthesis of T7 lysozyme in cell-free protein-synthesizing system from T7-infected F⁻ and F⁺ cells. T7 late protein-synthesizing activity of IS₃₀ from T7-infected F⁻ and F⁺ cells was compared. For this purpose, T7 late mRNA isolated from T7-infected F⁻ cells 10 min after infection was used as the lysozyme messenger to direct in vitro synthesis of T7 lysozyme, an easily assayable T7 late protein. To eliminate endogenous T7 lysozyme activity in the cell-free extracts from T7-infected cells, both F⁻ and F⁺ cells were infected with a mutant phage T7 13a, and IS₃₀ were prepared from T7 13a-infected cells.

The results shown in Table 1, which measured the activity of T7 lysozyme synthesized in vitro in the presence of T7 late mRNA, demonstrated a clear difference between the proteinsynthesizing activity of the cell-free systems prepared from T7-infected F⁻ and F⁺ cells harvested 10 and 17 min after infection. There was only a slight reduction in the lysozyme-synthesizing activity of the systems from F⁻ cells 10 and 17 min after infection, whereas the cell-free systems from F⁺ cells lost the activity greatly when T7 infection proceeded to the late period, 10 and 17 min after infection. The observed great reduction in protein-synthesizing activity of T7-infected F⁺ cells was not specific for T7 late mRNA. As described in detail in a separate paper (Yamada and Nakada, submitted for publication), the cell-free systems from T7-infected F⁺ cells showed a greater reduction in the activity to synthesize MS2 phage proteins in the presence of MS2 phage RNA and also T7 early proteins in the presence of T7 early mRNA compared to the systems from T7-infected F⁻ cells. The reduction in the activity of cell-free systems from T7-infected F⁺ cells was very similar to that shown here.

From the results presented above, it is clear that the protein-synthesizing machinery of T7-infected F⁺ cells nonspecifically deteriorates much faster than that of F⁻ cells, and this reduced protein-synthesizing activity in F⁺ cells late in T7 infection, as observed in vivo (Fig. 3B) and in vitro (Table 1), should contribute to the great reduction in the amount of T7 late proteins. In addition, as described in the previous section, F⁺ cells contain only about one-half of translatable T7 late mRNA compared to that contained in F⁻ cells. Therefore, it appears that both reduced amount of T7 late mRNA and the reduced activity of protein-syn-

![Graph](image)

**Fig. 11.** T7 lysozyme synthesis in T7-infected F⁻ and F⁺ cells. At indicated times after T7 infection, aliquots of the culture were withdrawn and sonically treated cell-free extracts were prepared as described. The activity of T7 lysozyme in each extract was assayed as described in Materials and Methods.

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* T7 late mRNA isolated from F⁻ cells 10 min after T7 infection was used to direct in vitro synthesis of T7 lysozyme. In vitro protein-synthesizing systems contained IS₃₀ prepared from T7 13a (gene 3.5 amber mutant)-infected F⁻ and F⁺ cells harvested at times indicated. After an incubation at 37 C for 45 min, 0.02 ml of the protein-synthesizing mixture was assayed for the radioactivity of ³H released from [³H]diaminopimelic acid-labeled E. coli cell wall material as described in Materials and Methods.
thesizing machinery of T7-infected F" cells are together responsible for the great reduction in the amount of T7 late proteins, thus accomplishing the status of restriction of T7 development.

DISCUSSION

Experiments described in this paper were designed to determine quantitative differences in the amount of T7-specific RNA and protein produced in T7-infected F" and F" cells to explain the restriction of T7 phage by F-factor-carrying E. coli strains. DNA-RNA hybridization was used to measure the amount of total T7-specific RNA in wild-type T7-infected cells and T7 early mRNA in T7 am27-infected cells. Measurement of T7 late mRNA was accomplished by assaying the messenger activity of the RNA to direct in vitro synthesis of T7 lysozyme. T7-specific RNA polymerase and T7 lysozyme, representing T7 early protein and late protein, respectively, produced in T7-infected cells, were assayed to quantitate the amount of T7-specific proteins.

The results presented here provided evidence that the abortive infection of F" cells by T7 phage cannot be explained by a defect in any single step of macromolecule synthesis and that the defective development of T7 included significant reductions in the amount of RNA and protein both early and late in T7 infection. In T7-infected F" cells, the amount of (i) T7 early mRNA is reduced to about 75 to 80% of that in F" cells; (ii) T7 early protein, measured by T7-specific RNA polymerase, is reduced to 75 to 80% of that in F" cells; (iii) T7 late mRNA is reduced to about one-half of that in F" cells; (iv) T7 late protein, measured by T7 lysozyme, is reduced to less than 10% of that in F" cells. In addition, we found that (v) the host shut-off function of T7 in F" cells is slow and incomplete; and (vi) the protein-synthesizing activity of cell-free extracts from T7-infected F" cells is greatly reduced compared to extracts from T7-infected F" cells.

The slight (20 to 25%) but significant reduction in the amount of T7 early mRNA and early protein in T7-infected F" cells should not be overlooked, because the slight reduction may have a cascading effect on the synthesis of greatly reduced amount of T7 late mRNA which is transcribed by the T7-specific RNA polymerase, one of the T7 early proteins. Furthermore, the observed incomplete host shut-off in T7-infected F" cells may also affect the amount of T7 late gene products by interfering with transcriptional and translational steps of T7 late gene expression.

However, the reduced amount of T7 late mRNA, only about one-half of the level in F" cells, and the greatly reduced activity of protein-synthesizing machinery, observed both in vivo and in vitro, of F" cells combined together seem to be the major, but not the sole, factors contributing to the insufficient production of T7 late proteins in F" cells, thus resulting in an abortive infection.

Although the defective protein-synthesizing system of T7-infected F" cells appears to play a major role in the restriction of T7, the defect is not specific for T7 late mRNA. The translational activity of cell-free systems from T7-infected F" cells decays much faster following the progress of T7 infection compared to that from F" cells, not only in T7 late mRNA-directed protein synthesis but also in MS2 RNA- and T7 early mRNA-directed synthesis, as described elsewhere (Yamada and Nakada, submitted for publication). Therefore, we conclude that specific translational control is not involved in the restriction of T7 in F" cells, and that a nonspecific general deterioration of protein-synthesizing machinery of T7-infected F" is a major contributing factor to the abortive T7 infection together with the reduced amount of T7 late mRNA.

Recent findings in two laboratories that the infection of F" cells with T7 results in a change in permeability of the infected cells should be discussed in relation to the results presented in this paper. Condit (5) found an increased permeability and decreased ATP level in T7-infected male cells. Britton and Haselkorn (2) also found that in T7-infected male cells a sudden loss of nucleotide pools and of ability to accumulate amino acids occurs due to a change in permeability. Since Condit and Steitz (6) and Britton and Haselkorn (2) observed a general cessation of RNA and protein synthesis at about 8 min of T7 infection of male cells, which correlates in time with the change of permeability, they interpreted the results as an indication that the restriction of T7 by F factor does not require any modification or inhibition of transcriptional and transcriitional apparatus of the cell.

Our results presented here show a good agreement with their results and interpretations in regard to the following. (i) Not only T7 late protein synthesis is blocked or reduced but also T7 late mRNA synthesis is reduced in T7-infected male cells, and therefore (ii) a class-specific translational control, as proposed before (12), is not responsible for the abortive infection of F" cells by T7.

Although we recognize the importance of the change in permeability occurring in T7-infected male cells which could explain the abortive infection by T7 due to the cessation of T7 RNA and protein synthesis, we observed that the in
vitro protein-synthesizing systems prepared from the F+ cells late in T7 infection were defective in protein synthesis even in the presence of sufficient quantities of nucleotides and amino acids. Our direct examination of the activity of cell-free systems from T7-infected F+ cells is not in accord with the indirect test by Condit and Steitz (6) which indicated that the translational machinery of T7-infected male cells is as competent as that from F- cells. However, it is possible that the change in permeability may cause a permanent, irreversible damage to the cells' protein synthesis machinery.

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