Evidence for the Presence of Nontranslated T7 Late mRNA in Infected F'(PIF⁺) Episome-Containing Cells

DAPHNE D. BLUMBERG AND MICHAEL H. MALAMY

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111

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The inability of T7 to develop in cells of *Escherichia coli* containing F⁺ or substituted F' episomes is a result of the failure to synthesize late proteins; no in vivo translation of mRNA species synthesized by the T7 RNA polymerase occurs. Further experiments have been performed to measure the amount of late mRNA in T7-infected F'(PIF⁺) cells. (We have designated the property of phage inhibition of F factors as PIF; the wild-type episome is therefore F'(PIF⁺). T7 late proteins were synthesized in vitro by using a system programed with RNA extracted from T7-infected F⁻ and F'(PIF⁺) cells. The T7 lysozyme, product of gene 3.5, and the gene 10 head protein were assayed. The following results were obtained: (i) mRNA capable of supporting in vitro synthesis of lysozyme and the gene 10 head protein is present in T7-infected F'(PIF⁺) cells; (ii) lysozyme mRNA extracted from T7-infected F'(PIF⁺) cells is present at 70 to 75% of the level found in T7-infected F⁻ cells; (iii) gene 10 mRNA is present at 35 to 78% of the level found in T7-infected F⁻ cells. No in vivo synthesis of either lysozyme or gene 10 protein can be detected in T7-infected F'(PIF⁺) cells although normal synthesis of these proteins occurs in F⁻ cells. These findings confirm that the block in T7 development in F'(PIF⁺) cells results from the failure to translate late classes of T7 RNA.

The development of bacteriophage T7 is inhibited in strains of *Escherichia coli* carrying either F⁺ or substituted F' factors such as F' lac (4, 7, 11). The F factors have two genes, designated *pifA* and *pifB*, which are responsible for T7 inhibition; episomes wild type in these genes are designated as F'(PIF⁺). Only the earliest class of T7 proteins, class 1, are made in the infected F'(PIF⁺) cells (11). By using methods such as polyacrylamide-agarose gel electrophoresis, DNA-RNA hybridization, and hybridization competition, it has been established that the late species of T7 RNA are present in T7-infected F'(PIF⁺) cells (3; T. Morrison, M. Malamy, M. Linial, and W. C. Summers, unpublished data). These methods have not, however, answered the question of whether the RNA synthesized in the infected F'(PIF⁺) cells has the same apparent stability of RNA synthesized in T7-infected F⁻ cells (19). In addition, it has not been conclusively established that the late T7 mRNA found in the infected F'(PIF⁺) cells is present in a translatable form. Summers and Jakes (20) attempted to resolve these questions by assaying the ability of RNA extracted from T7-infected F'(PIF⁺) cells to support the synthesis of T7 lysozyme in an in

vitro protein-synthesizing system. Although they were able to show that mRNA coding for lysozyme was present in normal amounts and in translatable form in the T7-infected F'(PIF⁺) cells, the interpretation of their experiments is subject to several reservations. First, the gene for lysozyme designated 3.5 (17) maps sufficiently close to the host RNA polymerase terminator site at position 20.2 (17) so that a significant portion of the lysozyme message translated in vitro could result from host RNA polymerase reading through the terminator site into the lysozyme gene. Second, lysozyme is a small protein, 13,000 daltons, and substantial degradation of the T7 RNA might occur in the infected F'(PIF⁺) cells without significant damage to the in vitro messenger capacity of a molecule as small as that coding for lysozyme.

We have attempted to circumvent these objections by assaying the capacity of T7 RNA extracted from infected F'(PIF⁺) cells to support the in vitro synthesis not only of T7 lysozyme, but also of one of the components of the phage virion, the product of gene 10. Gene 10 codes for the major component of the phage head, a protein weighing 38,000 daltons (17). By using a radioimmune co-precipitation assay for

the gene 10 head protein, we have measured the amount of gene 10 mRNA in T7-infected F⁻ and F'(PIF⁺) cells. These experiments have established the presence of normal amounts of translatable lysozyme and gene 10 mRNA in both F⁻ and F'(PIF⁺)-infected cells even though no synthesis of lysozyme or gene 10 head protein can be detected in the T7-infected F'(PIF⁺) cells *in vivo*.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. RV (F⁻) and RV/F⁻lac-W4680 (PIF⁺) have been described (8). Wild-type bacteriophage T7, T7 am193 am27 (gene 1), and T7 am13 (gene 10) are from the collection of F. W. Studier.

Media and buffers. Bacteria to be phage infected were grown in BLST minimal medium (11) supplemented with glucose and Casamino Acids as indicated. Bacteria to be used for the preparation of cell-free extracts were grown in ML broth (10).

Phosphate-buffered saline contained 0.01 M sodium phosphate buffer, pH 7.2, and 0.15 M NaCl. Sonication buffer contained 0.05 M Tris, pH 8, 0.01 M MgCl₂, 0.0001 M dithiothreitol, and 5% glycerol. RNA buffer contained 0.01 M NaCl, 0.01 M Tris, pH 7.5, 0.005 M MgCl₂, and 300 μ g of lysozyme per ml. Standard buffer contained 0.01 M Tris, pH 7.8, 0.06 M NH₄Cl, 0.01 magnesium acetate, and 0.006 M 2-mercaptoethanol.

Materials. [³H]lysine (20 Ci/mmol), [³⁵S]methionine (122 Ci/mmol), Protosol, and Liquifluor were from New England Nuclear Corp. DNase (RNase free) was from Worthington Biochemical Corp. Goat anti-rabbit gamma globulin antiserum was obtained from Miles Laboratories, Inc. ATP and lysozyme were purchased from Sigma Chemical Corp., and GTP and phosphoenolpyruvate were obtained from Calbiochem. Sephadex G25 (medium) was from Pharmacia. T7 antiserum was prepared as described by Adams (1).

[³⁵S]methionine labeling of proteins *in vivo* and preparation of crude extracts for assay of the gene 10 head protein. Cells were grown at 30 C in BLST containing 1% glucose, to a density of 3×10^8 cells/ml. The cells were UV irradiated under standard conditions (18). After an additional 30-min incubation at 30 C, the cells were infected with T7 at a multiplicity of 15. Samples (20 ml) were removed at several times during infection and incubated with 50 μ Ci of [³⁵S]methionine (122 Ci/mmol) for 2 min. Incorporation of radioactivity and further phage development were arrested by pouring the cells onto crushed, frozen media. Cells were collected by centrifugation at $10,000 \times g$ for 10 min, resuspended in 2 ml of chilled sonication buffer, and disrupted by sonication. The crude extracts were centrifuged at $10,000 \times g$ for 10 min and stored at 4 C.

Extraction of T7 RNA. A modification of the hot phenol procedure described by Salser et al. (13) was used. Cells were grown at 30 C in BLST supplemented with 1% glucose and 0.5% Casamino Acids to a density of 3×10^8 cells/ml and infected with T7 at a

multiplicity of 15. At the indicated times after infection, 25 ml of cells were poured onto 10 ml of crushed, frozen media and collected by centrifugation at $10,000 \times g$ for 10 min at 4 C. Each pellet was resuspended in 4 ml of RNA buffer and frozen in an acetone-dry ice bath. After thawing, the suspensions were made 1% in sodium dodecyl sulfate (SDS) and incubated at 37 C until clearing occurred. Sodium acetate buffer, pH 5.2, was then added to a final concentration of 0.1 M. The samples were heated at 65 C for 2 min in the presence of an equal volume of water-saturated phenol. The layers were separated by low-speed centrifugation at room temperature, and the aqueous layer was re-extracted four times at 37 C with equal volumes of water-saturated phenol. The aqueous layer was made 2 M in NaCl, and the RNA was precipitated by the addition of 1.5 volumes of 95% ethanol. After overnight storage at -20 C, the precipitate was collected by low-speed centrifugation at 4 C. The RNA was reprecipitated from 0.15 M NaCl with 1.5 volumes of 95% ethanol three additional times. The final RNA precipitate was washed with ether, dried under a stream of nitrogen, and resuspended in 0.2 ml of sterile water. RNA yields varied from 300 to 400 μ g per 25 ml of infected cells. Equal yields of RNA were obtained from T7-infected F⁻ and F'(PIF⁺) cells. All RNA concentrations were determined from the absorbance at 260 nm, with 1 optical density unit equal to 40 μ g/ml.

Preparation of cell-free extracts. The methods used were modified from the procedures described by Nirenberg and Matthaei (12) and Lodish and Robertson (6). Cell extracts were prepared from uninfected *E. coli* K-12 (RV) cells grown in ML broth and harvested in mid-log phase. Four grams of frozen cells were resuspended in 7 ml of standard buffer and disrupted in a French pressure cell at 13,000 to 14,000 lb/inch². The lysate was incubated at 4 C for 30 min after the addition of 0.5 μ g of DNase (RNase free) per ml. Cell debris was removed by low-speed centrifugation at 4 C. The resulting supernatant was centrifuged at $30,000 \times g$ for 30 min at 4 C. The S30 supernatant was incubated for 30 min at 30 C in a reaction mixture containing 0.25 mM of the 20 amino acids, 80 mM Tris, pH 7.8, 8 mM magnesium acetate, 50 mM NH₄Cl, 4 mM 2-mercaptoethanol, and 1.5 mg of ATP and 0.5 mg of phosphoenolpyruvate per ml. The preincubated S30 fraction was then passed through a column of Sephadex G25 (medium) washing with standard buffer. The turbid fractions were pooled, and samples were frozen in acetone-dry ice and stored until use at -70 C. All experiments to be described were repeated on two independently prepared S30 fractions.

Cell-free protein synthesis. Cell-free protein synthesis was carried out as described by Lodish (5) and Lodish and Robertson (6). Reaction mixtures contained 8.9 mg of an uninfected *E. coli* preincubated S30 fraction per ml, 55 mM ammonium cacodylate, pH 7.0, 110 mM NH₄Cl, 6.6 mM magnesium acetate, 11 mM 2-mercaptoethanol, 2.2 mM ATP, 4.6 mM phosphoenolpyruvate, 0.33 mM GTP, and 0.11 mM each of the 20 amino acids or, where indicated, 0.11 mM of each of 19 unlabeled amino acids and 55 μ Ci of

[^3H]lysine (20 Ci/mol) per ml. The amount of RNA added and the conditions of incubation are indicated in the figure legends.

Subsequent treatment of the reaction mixture depended upon the type of assay to be performed. (i) Determination of [^3H]lysine incorporation into trichloroacetic acid-precipitable material (total protein) has been described. (5). (ii) For assay of the gene 10 head protein by using the radioimmune co-precipitation reaction described below, a 0.2-ml reaction mixture was made 0.1 N in NaOH, incubated for 10 min at 37 C, and then chilled, neutralized with HCl, and diluted to a final volume of 1.2 ml in phosphate-buffered saline. The mixture was then centrifuged at $100,000 \times g$ for 105 min at 0 C to remove membrane and cell wall debris which interfere with the immune co-precipitation reaction. The S100 supernatant was used directly for the immune co-precipitation assay. (iv) In order to assay T7 lysozyme activity synthesized in vitro, samples of the reaction mixtures were used without further treatment.

Assay of the T7 gene 10 head protein. The T7 gene 10 head protein was assayed by a radioimmune co-precipitation reaction adapted from the procedures described by Tsugita et al. (21) and Wilhelm and Ginsberg (22). For proteins synthesized in vitro, a sample of the S100 supernatant was brought to a final volume of 1.0 ml with phosphate-buffered saline and made 0.05% in SDS, and 10 μl of rabbit anti-T7 antiserum ($K = 4,000$ [1]) was added. For proteins made in vivo, a sample of the crude extract containing [^{35}S]methionine-labeled proteins (described above) was treated in the same manner. Each mixture was vortexed vigorously and incubated for 2 h at 37 C, after which 0.1 ml of a dilution of goat anti-rabbit immunoglobulin G antiserum (previously determined to give optimal antigen precipitation) was added. Incubation was continued at 37 C for an additional 2 h, and the antigen-antibody complexes were collected by low-speed centrifugation. The precipitates were washed three times with cold 0.15 M NaCl, and the final precipitate was dissolved in 0.1 N NaOH. After a further 10-min incubation at 37 C, the complexes were precipitated with cold 5% trichloroacetic acid and boiled for 10 min. The precipitates were collected by low-speed centrifugation, washed with 5% trichloroacetic acid containing 2.5% Casamino Acids, and then washed with acetone. The final pellets were resuspended in electrophoresis sample buffer and boiled for 1 min before application to the polyacrylamide gels.

Polyacrylamide gel electrophoresis. Disc gels containing 7.5% polyacrylamide, 0.1% SDS, and 8 M urea were prepared as described by Morrison and Lodish (9). Electrophoresis was for 18 h at 5 mA per gel. The gels were frozen in 100% glycerol at -70 C and sliced into 1-mm fractions. Each fraction was incubated overnight at 37 C in 5 ml of toluene-Liquifluor containing 6% Protosol before being counted in a Beckman LS235 scintillation counter.

Assay of T7 lysozyme activity. T7 lysozyme activity was assayed as described by Schweiger and Gold (16) and in the accompanying paper (8) by measuring the enzyme-mediated excorporation of

[^3H]diaminopimelic acid from bacterial cell walls fixed on filter pads. Enzyme for the reaction was a sample of either an in vitro reaction mixture described above or a crude extract of T7-infected cells (8). The amount of enzyme for each assay was determined to be within the linear range of the lysozyme reaction. One enzyme unit releases 1 pmol of [^3H]diaminopimelic acid into 1 ml of ammonium acetate per 0.1 ml of in vitro reaction mixture in a standard reaction time of 40 min at 37 C.

RESULTS

Stimulation of protein synthesis by T7 RNA. The addition of RNA extracted from T7-infected F^- cells to a cell-free protein-synthesizing extract prepared from the uninfected *E. coli* K-12 strain RV led to a 10- to 15-fold stimulation of protein synthesis as measured by the formation of trichloroacetic acid-insoluble radioactivity. The rate of polypeptide synthesis under these standard conditions increased linearly until RNA concentrations in excess of 160 $\mu\text{g}/\text{ml}$ were used (Fig. 1). Above this concentration, the increase in rate was no longer linear.

Identification of the T7 gene 10 head protein synthesized in vivo or in vitro. The T7 gene 10 head protein was assayed by the radioimmune co-precipitation reaction described in Materials and Methods. Very similar patterns were obtained whether the T7 virion proteins were synthesized in vivo (Fig. 2A) or in vitro (Fig. 2C). The two major peaks present in

FIG. 1. Stimulation of protein synthesis by T7 RNA. Standard reaction mixtures (50 μl) contained [^3H]lysine and the indicated concentrations of T7 RNA extracted from infected F^- cells 10 min after infection. Incubation was at 30 C for 30 min.

FIG. 2. Polyacrylamide gel electrophoresis of proteins radioimmune co-precipitated with T7 antiserum. (A) T7 virion proteins made in vivo. Crude extracts were prepared from T7-infected F^- cells labeled with [^{35}S]methionine between 13 and 15 min after infection. Samples (0.1 ml) were taken for the radioimmune co-precipitation assay. Results are expressed as counts per minute per milligram of protein in the crude extract. (B) T7 virion proteins made in vivo after infection with an amber mutant in gene 10. Crude extracts were made from F^- cells infected with T7am13 mutant in gene 10 and labeled between 13 and 15 min after infection with [^{35}S]methionine. Samples (0.1 ml) were taken for the co-precipitation assay. Results are expressed as in (A). For T7 proteins made in vitro, standard reaction mixtures (0.2 to 0.4 ml) contained [^3H]lysine and 160 μg of the indicated RNA per ml. Incubations were at 30 C for 45 min. Samples (0.2 ml) were assayed by the radioimmune co-precipitation reaction. The graphs represent the distribution of [^3H]lysine per gel. (C) T7 total RNA extracted from T7-infected F^- cells at 10 min after infection. (D) T7 double gene 1 RNA extracted from T7-infected F^- cells at 10 min after infection. (E) No RNA.

panels A and C were not present upon immune co precipitation of T7 virion proteins made in vivo after infection with an amber mutant in gene 10 (Fig. 2B). On the basis of this result, we

conclude that the major proteins found in vivo are the products of gene 10, the major protein component of the phage head. The two peaks probably represent the gene 10 precursor protein (45,000 daltons) and the mature gene 10 protein (38,000 daltons). Both gene 10 products are found in the phage head (17). No proteins were detected by this assay when RNA was omitted from the in vitro protein-synthesizing system (Fig. 2E) or when the system was programed with RNA extracted from cells infected with an amber mutant in gene 10, the T7 RNA polymerase (Fig. 2D). This indicates that the major protein peaks synthesized in vitro and detected by this assay were specific products of T7 late mRNA. In addition, 50 to 90% of the material found in the two major protein peaks synthesized in vitro disappeared when the in vitro protein-synthesizing system was programed with RNA extracted from cells infected with an amber mutant in gene 10. Since the gel patterns of the immune co-precipitated proteins synthesized in vitro were very similar to those obtained in vivo, we can identify the two major peaks found in vitro as gene 10 proteins.

Stimulation of lysozyme and gene 10 head protein synthesis by T7 RNA. For the in vitro synthesis of lysozyme and the gene 10 head protein, conditions were adjusted so that the amount of enzyme activity synthesized was linearly dependent upon the amount of RNA added to the system.

Lysozyme production as a function of the concentration of T7 RNA used to program the cell-free, protein-synthesizing system was linear up to RNA concentrations of 160 $\mu\text{g}/\text{ml}$ (Fig. 3). The same result is seen for T7 gene 10 head protein synthesis as a function of the quantity of RNA added to the cell-free system (Fig. 4). All subsequent reactions for in vitro synthesis of lysozyme or the gene 10 head protein were done with RNA concentrations as close as possible to 160 $\mu\text{g}/\text{ml}$, since this concentration allows synthesis of the maximal amount of lysozyme and gene 10 head protein while still remaining in the linear range of the reaction. Since the amount of lysozyme or gene 10 head protein synthesized is proportional to the amount of RNA added to the in vitro protein synthesizing system, one can compare the fraction of T7 lysozyme or gene 10 head protein mRNA present in the RNA extracted from the T7-infected F^- and $F'(PIF^+)$ cells.

In vitro synthesis of lysozyme from RNA extracted from T7-infected F^- and $F'(PIF^+)$ cells. To determine the amount of T7 mRNA coding for lysozyme (gene 3.5) in T7-infected F^-

FIG. 3. *In vitro* lysozyme synthesis as a function of total RNA concentration. Standard reaction mixtures (0.1 ml) contained the indicated concentrations of T7 RNA extracted from infected F^- cells 10 min after infection. Incubation was at 30 C for 45 min. Samples (50 μ liters) were removed and assayed for lysozyme activity as described.

FIG. 4. T7 gene 10 head protein synthesis as a function of total RNA concentration. Standard reaction mixtures (0.2 to 0.4 ml) contained [3 H]lysine and the indicated concentrations of total RNA extracted from T7-infected F^- cells at 10 min after infection. Incubation was at 30 C for 45 min. Samples (0.2 ml) were assayed for [3 H]lysine incorporation into T7 gene 10 head protein by the radioimmune co-precipitation reaction. The co-precipitated proteins were subjected to polyacrylamide gel electrophoresis, and the radioactivity in the gene 10 peak regions was calculated and plotted as a function of the RNA concentration used to program the *in vitro* system.

and $F'(PIF^+)$ cells, RNA was extracted at several times after infection and used to program the *in vitro* protein-synthesizing system. Figure 5 shows the results of assaying the amount of

lysozyme synthesized in the *in vitro* system as a function of the time of RNA extraction. The RNA extracted from the T7-infected $F'(PIF^+)$ cells showed almost as much lysozyme-stimulating activity as did the RNA extracted from T7-infected F^- cells. These results are in agreement with the earlier report of Summers and Jakes (20) working with similar strains. Thus, the RNA coding for lysozyme is present in translatable form in T7-infected $F'(PIF^+)$ cells; however, no *in vivo* lysozyme synthesis can be detected in the T7-infected $F'(PIF^+)$ cells. It can be seen that even at 16 min, when lysozyme activity had reached a maximum in the F^- cell, only barely detectable amounts of lysozyme activity could be found in the infected $F'(PIF^+)$ cells (Fig. 5 of ref. 8).

***In vitro* synthesis of T7 gene 10 head protein from RNA extracted from T7-infected F^- and $F'(PIF^+)$ cells.** RNA was extracted from T7-infected F^- and $F'(PIF^+)$ cells at several times after infection and was used to program the *in vitro* protein-synthesizing system. The proteins made *in vitro* were then assayed for the presence of the T7 gene 10 head protein by the radioimmune co-precipitation reaction described in Materials and Methods. Figure 6 presents a series of graphs representing the distribution of radioactivity of the immune co-precipitated proteins from the *in vitro* reac-

FIG. 5. *In vitro* synthesis of lysozyme from RNA extracted from T7-infected F^- and $F'(PIF^+)$ cells. Standard reaction mixtures (0.1 ml) contained RNA (145 to 180 μ g/ml) extracted from either T7-infected F^- or $F'(PIF^+)$ cells at 5, 7, 9, 11, 13, 15, and 17 min after infection. Incubation was at 30 C for 45 min. Samples (50 μ liters) were removed and assayed for lysozyme activity. A background of 0.012 enzyme units of lysozyme made in the absence of added RNA was subtracted from each sample. Enzyme units are defined in Materials and Methods. Symbols: $\circ$, RNA extracted from T7-infected $F'(PIF^+)$ cells; $\bullet$, RNA extracted from F^- cells.

FIG. 6. T7 gene 10 head protein made *in vitro* from RNA extracted from T7-infected F^- and $F'(PIF^+)$ cells. Standard reaction mixtures (0.2 to 0.4 ml) contained [3H]lysine and RNA (145 to 180 μ g/ml) extracted from either T7-infected F^- or $F'(PIF^+)$ cells at 5, 7, 9, 11, 13, 15, and 17 min after infection. Incubation was at 30 C for 45 min. Samples (0.2 ml) were assayed by the radioimmune co-precipitation reaction and analyzed by polyacrylamide gel electrophoresis.

tions after electrophoresis in polyacrylamide gels. The samples differ only in the source and time of extraction of the RNA. Although the appearance of the gene 10 mRNA was delayed by about 2 to 4 min in the infected $F'(PIF^+)$ cells, the RNA extracted from these cells allowed synthesis of the gene 10 protein peaks, starting at 11 min and continuing through 17 min after infection. Thus, the mRNA for the gene 10 head protein was present in translatable form in the T7-infected $F'(PIF^+)$ cells; however, *in vivo* no gene 10 protein could be detected. F^- and $F'(PIF^+)$ cells were infected with T7 and incubated with radioactive amino acids at several intervals during infection. The cells were harvested and assayed for the gene 10 head proteins as described in Materials and Methods. Figure 7 shows the results of electrophoretic analysis of the immune co-precipitated proteins present in the T7-infected cells at four different time intervals. As can be seen, no gene 10 head protein was made *in vivo* in the T7-infected $F'(PIF^+)$ cells.

FIG. 7. T7 gene 10 head protein synthesized *in vivo* after T7 infection of F^- and $F'(PIF^+)$ cells. Crude extracts were prepared from T7-infected F^- and $F'(PIF^+)$ cells labeled with [^{35}S]methionine between 5 to 7, 7.5 to 9.5, 10 to 12, and 13 to 15 min after infection. Samples (0.1 ml) were taken for assay by the radioimmune co-precipitation reaction and analyzed by polyacrylamide gel electrophoresis. Results were normalized to radioactivity immune-co-precipitated per milligram of protein in the crude extract.

Translational capacity of RNA extracted from T7-infected $F'(PIF^+)$ cells. The reactions for the *in vitro* syntheses of lysozyme and the gene 10 head protein were performed so that the amount of lysozyme or gene 10 head protein synthesized would be directly proportional to the amount of total RNA added to the cell free system. As a result of these conditions, if the *in vitro* system were programed with equal amounts of total RNA extracted from T7-infected F^- and $F'(PIF^+)$ cells, the amount of lysozyme or gene 10 head protein synthesized *in vitro* could be used as a measure of the translational or messenger capacity of the RNA extracted from T7-infected $F'(PIF^+)$ cells when compared with the RNA from the infected F^- cells. These assays were performed with several independently extracted sets of RNA from T7-infected F^- and $F'(PIF^+)$ cells. Assigning a value of 100% to the amount of lysozyme or gene 10 head protein made from RNA extracted from T7-infected F^- cells, an equal amount of total RNA extracted from the T7-infected $F'(PIF^+)$ cells supported *in vitro* synthesis of 70 to 75% as much T7 lysozyme and 35 to 78% as much T7 gene 10 head protein (Table 1).

TABLE 1. *In vitro* translational capacity of RNA extracted from T7-infected F'(PIF⁺) cells

Exp no.	Conc of RNA ^a (μg/ml) in T7-infected:		Translatable mRNA in T7-infected F'(PIF ⁺) cells (% of F ⁻)	
	F ⁻	F'(PIF ⁺)	Lysozyme	Gene 10 ^a
1	172	180	75	34
2	175	172	76	
3	150	147	70	78
4	165	147		56
5	165	148		57

^a Determined from radioactivity in peak regions after polyacrylamide gel electrophoresis.

^b RNA was extracted from T7-infected F⁻ cells at 11 min after infection and from T7-infected F'(PIF⁺) cells at 13 min after infection. RNA extracted at these times allowed maximal synthesis of lysozyme and gene 10 protein (Fig. 5 and 6).

DISCUSSION

We have extracted the RNA from both F⁻ and F'(PIF⁺) cells at various times after T7 infection and used this RNA to program an *in vitro* protein-synthesizing system. Specific assays for two T7 late proteins, lysozyme and the gene 10 head protein, were performed on the products of the *in vitro* protein-synthesizing system. Our results clearly demonstrate that mRNA coding for T7 lysozyme and the gene 10 head protein is present in translatable form in T7-infected F'(PIF⁺) cells. These translatable messages were found in quantities up to 75% of that present in T7-infected F⁻ cells. Despite the presence of the T7 late mRNA in the infected F'(PIF⁺) cells, no synthesis of lysozyme or of the gene 10 head protein could be detected in these cells *in vivo*.

It is unlikely that degradation of the T7 mRNA in the infected F'(PIF⁺) cells is the explanation for the failure to synthesize lysozyme or the gene 10 head protein in these cells. The present study indicates that the functional half-lives of mRNA coding for lysozyme or the gene 10 head protein are at least as long in the infected F'(PIF⁺) cells as in the infected F⁻ cells, i.e., 2.5 to 3.5 min for lysozyme mRNA and 2.5 to 3 min for the gene 10 mRNA. These estimates, based on the results of three sets of experiments identical to those described in Fig. 5 and 6, assume that no extensive degradation of the RNA occurs during the extraction procedure. These functional half-lives, based on the functional capacity of the mRNA in the *in vitro* protein-synthesizing system, are considerably shorter than the average half-life of 20 min

reported by Summers (19), who used other methods for detecting T7 RNA. They are however, in agreement with the functional half-life for the gene 1 mRNA determined from experiments presented by Schleicher and Bautz (15). It is also unlikely that the 2-min delay in the appearance of gene 10 mRNA in the T7-infected F'(PIF⁺) cells is responsible for the failure to translate this message *in vivo*. First, we have observed the same delay in the appearance of late mRNA in mutants of the F factor which allow full synthesis of all the T7 proteins (unpublished data). Second, Studier (17) has reported that, in certain mutants of T7 which contain a deletion of the host RNA polymerase terminator site and an amber mutation in gene 1, there is significant synthesis of the late species of T7 RNA by the host RNA polymerase. Although the kinetics of appearance and amounts of these mRNA species are altered, translation of all the T7 proteins still occurs (2, 17). This result makes it unlikely that a simple change in the time of appearance or the amount of mRNA in the F'(PIF⁺) cells could lead to an absolute failure to translate this RNA. Thus, these experiments reaffirm the hypothesis that the block in T7 development in F'(PIF⁺) cells results directly or indirectly from the failure to translate late species of T7 mRNA.

Examination of polysome profiles in T7-infected F⁻ and F'(PIF⁺) cells has revealed that at late times after infection, when polysomes and 70S ribosomes are present in the T7-infected F⁻ cells, only 30S and 50S ribosomal subunits are present in the T7-infected F'(PIF⁺) cells (unpublished observation). Thus, the late species of T7 mRNA, which we have shown to be present in the T7-infected F'(PIF⁺) cells, do not seem to form 70S ribosomal initiation complexes. Indeed, evidence is accumulating which indicates that there are changes in the initiation factor fraction after T7 infection of F⁻ and F'(PIF⁺) cells (14; T. G. Morrison and M. H. Malamy, manuscript in preparation).

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