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T7 Protein Synthesis in F′ Episome-Containing Cells: Assignment of Specific Proteins to Three Translational Groups

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Synthesis of many T7 proteins is prevented in F′ episome-containing cells. In order to quantitate the degree of inhibition, we measured the activity of several T7 proteins in extracts prepared from T7-infected F⁻ and F′ cells and cells containing F factors mutant in phage inhibition [F′(PIF⁻2A) and F′(PIF⁻2A,2B)]. In addition, we were able to assign specific T7 proteins to the three translational units previously defined by polyacrylamide gel analysis of T7 proteins made in F⁻ and episome-containing cells. After T7 infection, the presence of the wild-type F′ (PIF⁺) episome led to greater than 90% inhibition of T7 DNA polymerase (product of gene 5), T7 lysozyme (gene 3.5), and gene 10 capsid protein synthesis. Nearly normal amounts of T7 RNA polymerase (gene 1) were made in these cells. T7 infection of cells containing the mutant F′ (PIF⁻2A) episome led to normal synthesis of T7 RNA polymerase and T7 DNA polymerase; T7 lysozyme was synthesized at 30% of the maximal level in these cells; T7 gene 10 capsid protein synthesis was inhibited by 90%, and T7 DNA synthesis was arrested in these cells. T7 infection of cells containing the mutant F′ (PIF⁻2A,2B) episome led to synthesis of normal levels of the enzymes assayed.

The development of phage T7 is inhibited in *Escherichia coli* K-12 cells which contain a wild-type F⁺ or substituted F′ episome (12). The F factor interferes with phage development by preventing the synthesis of all but the earliest (class 1) T7 proteins (11, 13). We have designated the property of phage inhibition by F factors as PIF; the wild-type episome is therefore F′ (PIF⁺). Two types of PIF⁻ F factor mutants defective in T7 inhibition have been isolated (13). Most of the mutants (87%), designated F′ (PIF⁻2A), allow partial T7 development as measured by phage burst size and plaque morphology. The second class of F factor mutants, designated F′ (PIF⁻2A,2B), allows normal T7 development. Analysis by polyacrylamide gel electrophoresis of proteins made after T7 infection of the PIF⁻ F factor mutants has revealed that (i) all PIF⁻ and PIF⁺ cells synthesize class 1 proteins; (ii) F′(PIF⁻2A) mutants also allow the synthesis of a second class of proteins designated 2A; and (iii) F′ (PIF⁻2A,2B) mutants allow the synthesis of all T7 proteins, including a third class designated 2B (13).

These observations allow the separation of T7 protein synthesis into three translational groups. We have assigned specific T7 proteins to each group by assaying the enzymatic activity or immunological reactivity of specific T7 proteins in extracts prepared from T7-infected F⁻, F′ (PIF⁺), F′ (PIF⁻2A), and F′ (PIF⁻2A,2B) cells.

These assays have also allowed the quantitation of the level of inhibition of class 2A and 2B protein synthesis in T7-infected F′ (PIF⁺) and F′ (PIF⁻2A) cells. In the presence of the F′ (PIF⁺) episome, less than 5% of T7 DNA polymerase, lysozyme, and gene 10 head protein are synthesized compared with the amounts present in T7-infected F⁻ cells. T7-infected F′ (PIF⁻2A) cells contain 73% of the T7 DNA polymerase activity, whereas gene 10 head protein synthesis is less than 10% of the level in F⁻ cells. T7-infected F′ (PIF⁻2A,2B) cells contain normal amounts of these proteins.

Even though class 2A and 2B proteins are not synthesized in T7-infected F′ (PIF⁺) cells, late T7 mRNA extracted from these cells can direct the synthesis of T7 class 2A and 2B proteins in cell-free systems (1, 20).

MATERIALS AND METHODS

Bacterial strains, episomes, and bacteriophages. Strains and episomes used in these experi-

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ments are listed in Table 1. Wild-type T7 was obtained from W. C. Summers and was from the collection of F. W. Studier.

Media and reagents. Bacteria were grown in BLST minimal medium (13). Rifampicin was obtained from Schwarz/Mann. Radioactive compounds obtained from New England Nuclear Corp. were [^{3}H]CTP (27.5 Ci/mmol), [^{3}H]dTTP (18 Ci/mmol), [^{3}H]thymidine (18 Ci/mmol) and [^{35}S]methionine (122 Ci/mmol). [^{3}H]diaminopimelic acid (DAP) (200 mCi/mmol) was obtained from Amersham/Searle. T7 DNA was extracted from purified virions (11).

Enzyme assays. T7 RNA polymerase was assayed by the procedure of Chamberlin et al. (3). Crude extracts prepared from T7-infected cells were the source of enzyme. Cells grown in BLST supplemented with 1% glucose and 1% Casamino Acids at 30 C to a cell density of 3×10^8 cells/ml were infected with T7 at a multiplicity of 15. Samples (25 ml) were removed at various times after infection and chilled on crushed, frozen medium to arrest phage development. Cells were collected by centrifugation at 4 C, resuspended in 2 ml of cold buffer A (3) and broken by sonication. The crude extracts were centrifuged at 10,000 $\times$ *g* for 10 min, and the supernatant was removed and stored at 4 C. The reaction mixture contained 20 μg of rifampicin per ml, 40 mM Tris-hydrochloride, pH 8.0, 20 mM $MgCl_2$, 0.24 mM ATP, GTP, and UTP, 0.2 μCi of [^{3}H]CTP, 10 mM 2-mercaptoethanol, 100 μg of bovine serum albumin, T7 DNA, and crude enzyme in a total volume of 0.15 ml. After incubation at 37 C for 10 min, the reaction was stopped with 1 ml of cold 5% trichloroacetic acid, and the samples were collected on glass fiber filters (Whatman GF/A). Because of varying amounts of T7 DNA already present in the enzyme preparations, enough substrate T7 DNA was added to each reaction mixture to ensure DNA saturation. Under the above conditions, 8.7 μg of T7 DNA per 0.15 ml of reaction mixture was determined to be adequate. One enzyme unit converts 1 pmol of [^{3}H]CTP into trichloroacetic acid-insoluble product in a reaction mixture of 0.15 ml during a standard incubation time of 10 min at 37 C.

The assay for T7 DNA polymerase, developed by Grippo and Richardson (6), measures the conversion of [^{3}H]deoxyribonucleoside triphosphates into trichloroacetic acid-insoluble material. Enzyme for this reaction was a crude cell extract prepared as outlined for RNA polymerase except that all bacterial strains used for this assay contained a *pol A*$^-$ mutation in order to reduce incorporation of radioactivity due to host enzymes. The reaction mixtures (0.15 ml) contained 88 mM potassium phosphate buffer, pH 7.4, 6.7 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.5 mM heat-denatured salmon sperm DNA (20 μg), 0.15 mM dCTP, dATP, and dGTP, crude enzyme, and 0.1 μCi of [^{3}H]dTTP. After incubation at 37 C for 30 min, the reaction was stopped by the addition of 1 ml of cold 0.5 M NaCl and 2 ml of 10% trichloroacetic acid. The precipitates were collected on glass fiber filters (Whatman GF/A) and prepared for scintillation counting. An enzyme unit catalyzes the incorporation of 10 nmol of labeled nucleotide into an acid-insoluble product during an incubation period of 30 min at 37 C.

The activity of T7 lysozyme was measured, as described by Schweiger and Gold (15), by the enzyme-mediated excorporation of radioactivity from [^{3}H]DAP-labeled cell-wall substrate fixed on filter pads. Enzyme for this reaction was a crude cell extract prepared as described for RNA polymerase. *E. coli* strain AT713, lacking the enzyme DAP-decarboxylase (15), is used to prepare [^{3}H]DAP-labeled cell-wall substrates (15). Enzyme reactions were carried out in vials containing one prepared filter pad and 0.5 ml of 0.1 M ammonium acetate. Enzyme was added in volumes of 5 to 50 μliters. At various times after addition of enzyme, samples of the ammonium acetate solution were removed and added immediately to scintillation vials for counting with 5 ml of Aquasol (New England Nuclear Corp.). One enzyme unit releases 1 pmol of [^{3}H]DAP into 1 ml of ammonium acetate in a standard reaction time of 30 min at 37 C.

Lysis of T7-infected cells. The kinetics of lysis of T7-infected cultures was measured by following the turbidity at 600 nm. All cultures were grown in BLST containing 1% glucose and 1% Casamino Acids at 30 C and infected with T7 at a multiplicity of 0.5 to 5 phage/cell during exponential growth when a cell density of 3×10^8 cells/ml had been obtained.

Total DNA synthesis. The rate of DNA synthesis

TABLE 1. *Strains and episomes*

Strain or episome	Genotype[a]	Source and reference
RV (references F$^-$)	F$^-$, *thi*$^-$, *lac*-Δ x 74	Pasteur Institute
RV-Pol A	F$^-$, *thi*$^-$, *lac*-Δ x 74, *pol* A$^-$	RV, rham$^-$ transduced with P1 grown on W3110P3478
W3110P3478	F$^-$, *thy*$^-$, *pol* A$^-$	R. Moses (4)
AT713	F$^-$, *lys*A$^-$,	E. Siegel
F'W4680 (reference PIF$^+$)	F'lac-Δ W4680, *pif*A$^+$, *pif*B$^+$	Pasteur Institute (13)
F'W6D (reference PIF$^-$2A)	F', *pif*A$^-$, *pif*B$^+$	Derived from F'W4680 (13)
F'E23D (reference PIF$^-$2A, 2B)	F', *pif*A$^-$, *pif*B$^-$	Derived from F'W4680 (13)

[a] The conventions of Demerec et al. (5) and the nomenclature incorporated in the latest *E. coli* map (21) were used to describe cell genotypes. Only markers relevant to this study are included. PIF$^+$ corresponds to the wild type F' episome; the basis for this convention and the designation of episomes mutant in this function is described in Morrison and Malamy (13).

in cells infected with T7 was measured by following the incorporation of [^{3}H]thymidine into trichloroacetic acid-precipitable material. Cells growing in BLST supplemented with 1% glucose and 1% Casamino Acids at 30 C were infected with T7 at a multiplicity of 15 to 20 phage/cell. Samples (1 ml) were removed at intervals during infection and incubated in the presence of 0.1 μCi of [^{3}H]thymidine and 12.5 μg of deoxyadenosine for 3 min at 30 C. Incubation was terminated by the addition of 1 ml of 10% trichloroacetic acid. The samples were collected on glass fiber filters (Whatman GF/A). The incorporation of radioactive thymidine during any pulse period is a measure of the average rate of DNA synthesis during that particular period of infection. Total synthesis up to any particular time during infection may be calculated by adding the radioactivity in all pulses up to that time.

T7 structural proteins. The T7 gene 10 capsid protein was measured in crude extracts made from T7-infected F$^-$, F′ (PIF$^+$), F′ (PIF$^-$2A), and F′ (PIF$^-$2A,2B) cells which had been radioactively labeled by incubation with [^{35}S]methionine between 13 and 15 min after infection at 30 C. Radioactive extracts were incubated with rabbit antiserum directed against T7 phage particles. The antigen-antibody complexes were precipitated by incubation with goat anti-rabbit immunoglobulin antiserum. The precipitates obtained were subjected to urea-sodium dodecyl sulfate disc gel electrophoresis, and the gels were sliced horizontally into 1-mm slices. Radioactivity in each slice was determined in a Beckman LS235 scintillation counter after the samples were incubated overnight at 37 C in toluene-Liquifluor containing 6% Protosol. Details of the assay procedure are included in the accompanying paper (1).

RESULTS

T7 RNA polymerase. T7 RNA polymerase, the product of gene 1, is one of the first proteins made after T7 infection. By comparison with previously published polyacrylamide gel patterns of T7 proteins (13, 19) we were able to conclude that all T7-infected cells (F$^-$, F′ [PIF$^+$], F′ [PIF$^-$2A], and F′ [PIF$^-$2A,2B]) allow synthesis of this protein. Assays of the enzyme activity in T7-infected cell extracts confirmed this assignment.

Extracts were prepared from T7-infected cells at several times after infection. The RNA polymerase activity present in each extract was assayed as outlined in Materials and Methods. The results (Fig. 1) are plotted in terms of the specific activity of the enzyme in each extract. All extracts made from 7.5 to 20 min after infection contained T7 RNA polymerase activity, whereas the 11-min sample contained maximal activity. Although RNA polymerase activity was detected in all cells, T7-infected F′ (PIF$^-$2A) and F′ (PIF$^+$) cells contained slightly less RNA polymerase activity than did infected

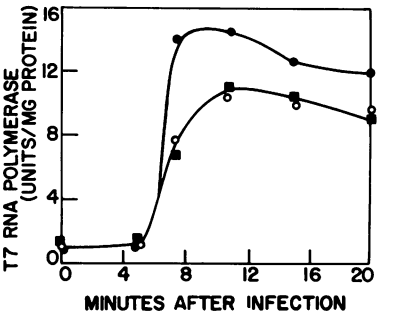

FIG. 1. *T7 RNA polymerase activity. T7 RNA polymerase activity was measured in crude extracts prepared from F$^-$ (●), F′ (PIF$^+$) (○), and F′ (PIF$^-$2A) (■) episome-containing cells infected with T7 at the times indicated. Enzyme units are defined in Materials and Methods.*

F$^-$ cells. In other experiments, however, there was a negligible difference in the enzyme units present in T7-infected F$^-$ and F′ (PIF$^-$2A) cells, whereas extracts from infected F′ (PIF$^+$) cells always contained slightly less T7 RNA polymerase activity, usually 75% of that found in the infected F$^-$ cells. Thus, despite the presence of the F′ (PIF$^+$) episome, T7 RNA polymerase was made in close to normal amounts.

DNA synthesis in T7-infected F$^-$ and F factor-containing strains. The protein products of genes 1.7 through 6 are necessary for T7 DNA synthesis (16, 17).

Polyacrylamide gel analysis of proteins made in T7-infected F′ (PIF$^+$) cells failed to demonstrate the presence of any of these proteins, whereas analysis of proteins made in T7-infected F′ (PIF$^-$2A) cells indicated that at least some of these proteins were synthesized. It was, therefore, of interest to characterize the properties of T7 DNA synthesis in infected F′ (PIF$^-$2A) cells. Total DNA synthesis in T7-infected F$^-$, F′ (PIF$^+$), F′ (PIF$^-$2A), and F′ (PIF$^-$2A,2B) cells was measured as described in Materials and Methods. Figure 2 shows the total synthesis of thymidine-containing material from the first time period, and the inset shows the average rate of incorporation during each 3-min interval. As has been shown by Linial and Malamy (11) with the related phage ϕII, infection of F$^-$ strains results in rapid incorporation of [^{3}H]thymidine throughout infection. Infection of F′ (PIF$^+$) cells leads to a cessation of thymidine incorporation early in infection. T7 infection yields similar results (Fig. 2). The inset in Fig. 2 shows the rate of incorporation of [^{3}H]thymidine in T7-infected F$^-$, F′ (PIF$^+$), and F′ (PIF$^-$2A) cells. T7 infection of F$^-$ cells resulted in a maximal rate of

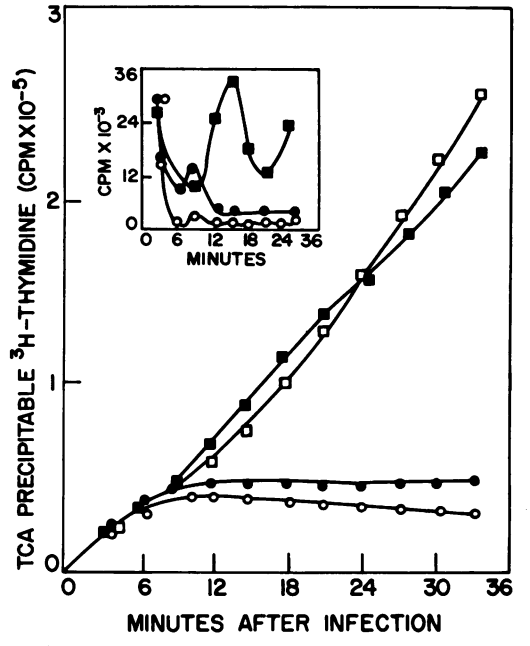

FIG. 2. DNA synthesis in T7-infected F$^-$- *and F factor-containing cells. The inset gives the average rate of DNA synthesis for each 3-min pulse period as measured by the incorporation of [^{3}H]thymidine into trichloroacetic acid-precipitable material. The large graphs show the total DNA synthesis in each culture calculated by summing the incorporation of trichloroacetic acid-precipitable ^{3}H in each 3-min interval previous to the point of infection in question. Uninfected cells* (□); *T7-infected cells, F$^-$*(■); *F' (PIF$^+$)* (○); *F' (PIF$^-$2A)* (●).

DNA synthesis at 12 to 18 min after infection at 34 C. After T7 infection of F' (PIF$^+$) cells, there was little incorporation of [^{3}H]thymidine after the first 3 min of infection. T7 infection of F' (PIF$^-$2A) cells resulted in a maximal rate of synthesis (disregarding the first 3 min) at 9 min after infection at 34 C, after which time synthesis stopped. This rate was 36% of the maximal rate observed in T7-infected F$^-$ cells.

T7 DNA polymerase. Even though T7 DNA synthesis appeared to be arrested after T7 infection of F' (PIF$^-$2A) cells, T7 DNA polymerase activity could be detected after T7 infection of these cells. No DNA polymerase activity, however, could be detected in T7-infected F' (PIF$^+$) cells. Crude extracts were prepared from T7-infected RVpolA$^-$ (F$^-$) and episome-carrying derivatives and assayed for T7 DNA polymerase activity. Figure 3 presents the results of these assays in terms of the specific activity of T7 DNA polymerase in each extract. Extracts prepared at 5 or 7.5 min after infection contained negligible activity. Extracts prepared from T7-infected F' (PIF$^+$) cells never contained significant DNA polymerase activity even at late times after infection. Both F' (PIF$^-$2A) and F$^-$ infected cells, however, contained significant amounts of T7 DNA polymerase, the activity of which reached a maximum at 15 min after infection. The maximal level of DNA polymerase acitivty in T7-infected F' (PIF$^-$2A) cells was about 73% of that found in infected F$^-$ cells.

Cell lysis and T7 lysozyme activity. Rapid culture lysis is another property of T7- and φII-infected F$^-$ cells (11, 13). When a growing F$^-$ culture was infected with T7 at a multiplicity of 0.5 or greater, growth of the culture ceased within 10 min and the cells lysed rapidly, liberating mature phage. T7-infected F' (PIF$^+$) cells also stopped growing, but the turbidity of the culture remained constant; the infected F' (PIF$^+$) cells did not lyse. Figure 4 presents a series of lysis curves obtained after infection of F$^-$ and episome-containing derivatives with T7. Infection of F' (PIF$^-$2A) cells led to a lysis pattern different from that of either F$^-$ or F' (PIF$^+$) cells. Instead of the rapid lysis characteristic of F$^-$ cells, only slow lysis occurred. In contrast, F' (PIF$^-$2A,2B) cells exhibited rapid culture lysis after infection, characteristic of infected F$^-$ cells. These results suggest that there are two components involved in cell lysis following T7 and φII infection. One of the components is linked to class 2A protein synthesis, and the second requires class 2B protein synthesis.

Assays for T7 lysozyme were performed to determine whether this protein could be identified as a class 2A or 2B activity. Figure 5 presents the results of these assays in terms of

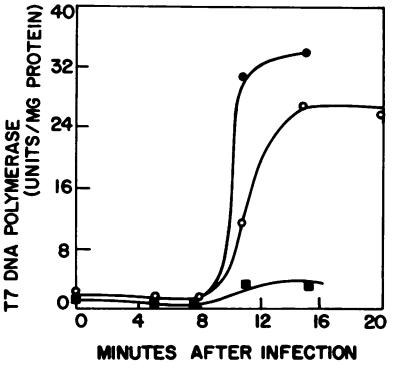

FIG. 3. *T7 DNA polymerase activity. Enzyme extracts were prepared from strain RV polA and episome-containing derivatives infected with T7 at the times indicated. F$^-$* (●); *F' (PIF$^+$)* (■); *F' (PIF$^-$2A)* (○).

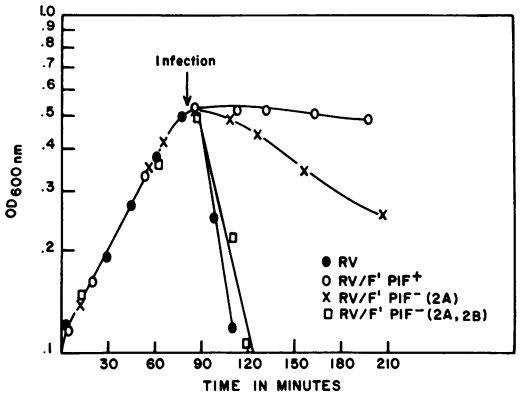

FIG. 4. *Culture lysis after T7 infection. Exponentially growing cultures at 34 C were infected, as indicated by the arrow, with T7 at a multiplicity of 0.5 phage/cell. (Similar results were obtained with a multiplicity of 5 phage/cell.) The turbidity was monitored at 600 nm in a Gilford spectrophotometer. F⁻ (●); F' (PIF⁺) (O); F' (PIF⁻2A) (×); F' (PIF⁻2A, 2B) (□).*

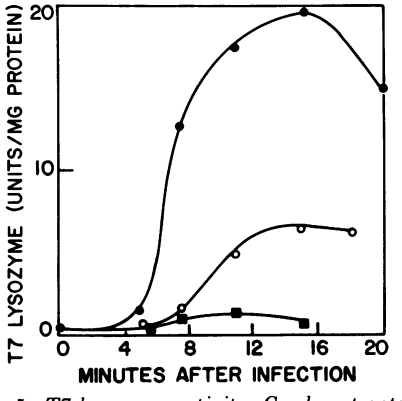

FIG. 5. *T7 lysozyme activity. Crude extracts were prepared from T7-infected F⁻- and episome-containing cells at the times indicated and assayed for T7 lysozyme as described in Materials and Methods: F⁻ (●); F (PIF⁺) (■); F (PIF⁻2A) (O).*

the specific activity of lysozyme in each extract. Extracts from T7-infected F⁻ cells contained lysozyme beginning at 7.5 min after infection (in other experiments, some lysozyme activity could be detected as early as 5 min after infection). Extracts from T7-infected F' (PIF⁺) cells contained negligible lysozyme activity even at late times after infection. Lysozyme activity was detectable in T7-infected F' (PIF⁻2A) cells, but in this experiment the maximal amount present was only 30% that found in T7-infected F⁻ cell extracts. In other experiments (data not shown), the amount of lysozyme activity in F' (PIF⁻2A) cells was as high as 75% of the F⁻ level. These results indicate that lysozyme may be classified as a 2A protein.

T7 virion protein synthesis. The major protein components of the T7 virion are synthesized late in infection (17). Previously, we have reported the absence of virion components after analysis of polyacrylamide gels of proteins synthesized in either T7-infected F' (PIF⁺) or F' (PIF⁻2A) cells (13). The same method of analysis demonstrated the presence of virion components in T7-infected F⁻ and F' (PIF⁻2A,2B) cells. We have developed a specific assay for the gene 10 head protein and have examined extracts of T7-infected cells for this protein. The F⁻ and episome-containing cells were infected with T7 and incubated with radioactive amino acids between 13 and 15 min after infection. Extracts prepared from these cells were assayed for the gene 10 head protein by a radioimmune co-precipitation reaction described in detail in the accompanying paper (1). Figure 6 shows the results of polyacrylamide gel electrophoresis of the immune co-precipitated proteins. Although T7-infected F⁻ and F' (PIF⁻2A,2B) cells allowed full synthesis of the gene 10 protein, this protein could not be detected in the T7-infected F' (PIF⁺) cells, and less than 10% of the maximal levels could be found in the infected F' (PIF⁻2A) cells. Since T7-infected F' (PIF⁻2A) cells did not synthesize significant amounts of the gene 10 product, this virion component must be classified as a 2B protein.

DISCUSSION

F factor inhibition of phage T7. We have demonstrated that, after T7 infection, the presence of the F' (PIF⁺) episome leads to inhibition of the synthesis of T7 DNA polymerase by 94%, T7 lysozyme by at least 95%, and T7 gene 10 head protein by 90%. In the accompanying paper (1), we present the results of assays which quantitate the amount of translatable T7 late mRNA present in the T7-infected F' (PIF⁺) cells. These results show that the deficiency in T7 protein synthesis in T7-infected F' (PIF⁺) cells cannot be explained by the absence of translatable mRNA. Similar studies have been reported by Summers and Jakes (20), who also demonstrated that mRNA isolated from T7-infected F' (PIF⁺) cells could direct the in vitro synthesis of lysozyme in a cell-free system although no lysozyme activity could be detected in F' (PIF⁺) cells in vivo. These results verify earlier conclusions that F factor inhibition of T7 development operates to indirectly or directly

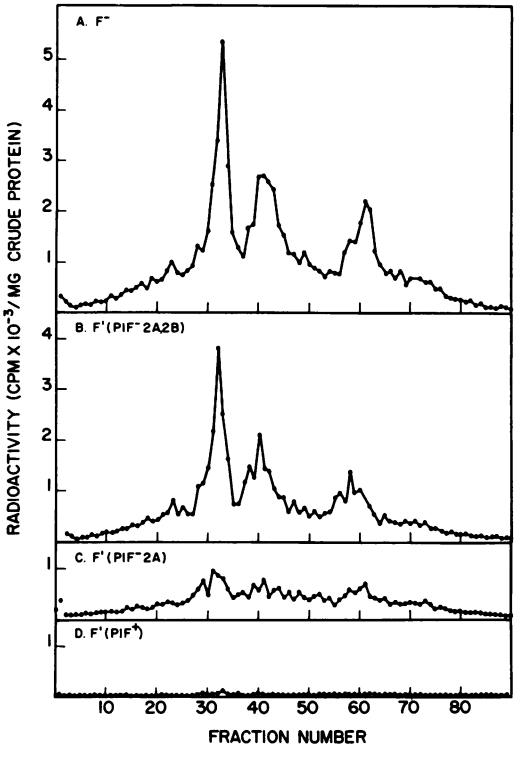

FIG. 6. *Gene 10 protein synthesis in vivo. T7-infected F⁻, F' (PIF⁻2A), F' (PIF⁻2A,2B), and F' (PIF⁺) cells were radioactively labeled with 2.5 μCi/ml of [³⁵S]methionine between 13 and 15 min after infection. Crude extracts were prepared and assayed for the gene 10 head protein by the radioimmune co-precipitation reaction described in the accompanying paper (1). The immune co-precipitated proteins were analyzed on polyacrylamide gels which were sliced into 1-mm fractions and counted as described in Materials and Methods. The results are normalized to milligrams of protein in the crude extract.*

prevent the translation of T7 late mRNA (10, 13).

T7 translational units. The results presented previously (13) and in this paper demonstrate that cells containing wild-type F' (PIF⁺) or (PIF⁻) episomes can be used to separate specific T7 proteins and functions into translational units. Furthermore, T7 infection of F' (PIF⁺) and F' (PIF⁻2A) cells allows studies of discrete stages of phage development in the absence of later stages. We can assign T7 RNA polymerase to class 1, T7 DNA polymerase and T7 lysozyme to class 2A, and T7 gene 10 protein to class 2B.

Studier and Maizel (19) and Studier (17) have also classified T7 proteins into three groups. Their class I proteins are defined as those proteins translated from early mRNA which is transcribed by the host RNA polymerase. Late proteins are subdivided into two classes, designated class II and class III, on the basis of their time of appearance during the T7 development cycle and the time their synthesis stops. Most of the class II proteins are involved in DNA metabolism, whereas class III proteins include virion components and maturation proteins.

Our results allow us to compare the three groups of T7 proteins defined by Studier with the three translational groups defined by studies with F' episome-containing cells.

It is clear, by direct assay of T7 RNA polymerase activity and by analysis of proteins on polyacrylamide gels, that Studier's class I proteins are identical to the class 1 proteins made in F⁻ and F' episome-containing cells. These two classes are also identical in the times of onset and termination of synthesis.

Studier has classified all proteins required for DNA synthesis and host cell DNA degradation (encoded by genes 1.7 through 6) as class II proteins. At first glance, it would appear that class II proteins correspond to class 2A proteins. Indeed, we have shown that T7 DNA polymerase, product of gene 5, is a class 2A protein. However, synthesis of 2A proteins, in the absence of class 2B synthesis, does not result in normal T7 DNA synthesis. In T7-infected F' (PIF⁻2A) cells, which synthesize 2A but not 2B proteins, DNA synthesis is arrested. Normal T7 DNA synthesis occurs only in F⁻ and F' (PIF⁻2A,2B) cells which synthesize all three classes of T7 proteins.

Several studies have shown (7, 16, 18) that mutation in any of the genes necessary for T7 DNA synthesis results in the arrest of DNA synthesis (DA phenotype) or the complete absence of T7 DNA synthesis (DO phenotype). By using the incorporation of [³H]thymidine into acid-insoluble material as a measure of DNA synthesis, it has been established that cells infected with wild-type T7 incorporate radioactivity at a maximal rate between 15 and 30 min after infection at 30 C. Cells infected with T7 DA mutants (mutant in genes 2, 3, or 6) incorporate [³H]thymidine between 10 and 20 min after infection, but the maximal rate of DNA synthesis in these cells is only one-third the rate measured after wild-type T7 infection. Cells infected with T7 DO mutants (mutants in genes 1, 4 or 5) incorporate [³H]thymidine at a low rate during the early minutes of infection and then cease incorporation. The results presented in this paper demonstrate that T7 infection of F' (PIF⁺) cells resembles infection of F⁻

cells by T7 DO mutants, and T7 infection of F′ (PIF⁻2A) cells resembles infection of F⁻ cells by T7 DA mutants. Since we have shown that T7-infected F′ (PIF⁺) cells contain negligible T7 DNA polymerase activity, the DO phenotype is expected. In addition, polyacrylamide gel analysis of T7 proteins made in T7-infected F′ (PIF⁺) cells suggests that all enzymes needed for DNA synthesis are missing.

Some proteins necessary for T7 DNA synthesis, including T7 DNA polymerase, are expressed in T7-infected F′ (PIF⁻2A) cells. However, some proteins essential for DNA synthesis must not be synthesized, since DNA synthesis is arrested in these cells. From previous studies on DA mutants of T7, the DA phenotype of T7-infected F′ (PIF⁻2A) cells might indicate the absence of one or more of the products of genes 2, 3, or 6. We have previously shown (13) that the combined activity of the gene 3 endonuclease (2) and the gene 6 exonuclease (8, 9, 14) cannot be detected after T7 infection of F′ (PIF⁻2A) cells. However, the presence of gene 3 activity in the absence of gene 6 activity could not be ruled out in those assays.

The lack of exonuclease or endonuclease activity, or both, in cells synthesizing 2A but not 2B proteins might be explained in several ways. Perhaps both proteins are made as class 2A proteins but their activity is dependent upon the synthesis of another protein belonging to class 2B. Alternatively, the gene 6 exonuclease may belong to the class 2B translational unit but might appear early enough to be included as a class II protein when groups of proteins are defined only by their sequential appearance in protein extracts assayed on polyacrylamide gels.

T7 lysozyme, product of gene 3.5, classified as a class II protein by Studier (17), is a class 2A protein since it can be synthesized in F′ (PIF⁻2A) cells. It is clear from the lysis curves presented in Fig. 4 that, in addition to T7 lysozyme, there is a 2B function necessary for rapid culture lysis after T7 infection. It is known that certain gene 3.5 amber mutants are able to lyse F⁻ cells, albeit slowly (17). The strongest evidence for a class 2B lysis function is the isolation of a lysis-defective mutant of T7 mapping at gene 17.5, which produces normal amounts of the gene 3.5 lysozyme activity (L. Blair, C. Kanter, and D. Botstein, personal communication).

The extent to which class III proteins, products of gene 7–19, are the same as class 2B proteins (those T7 proteins not translated in infected F′ [PIF⁻2A] cells) is not known. We have demonstrated that the gene 10 head protein is a class 2B protein. In addition, several other virion components have been assigned to class 2B on the basis of polyacrylamide gel analysis of proteins extracted from T7-infected F′ (PIF⁻2A) and F′ (PIF⁻2A,2B) cells.

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ADDENDUM IN PROOF

The T7 lysozyme, product of gene 3.5, referred to in this and the accompanying paper (1) has recently been identified as an *N*-acetylmuramyl-L-alanine amidase (M. Inouye, N. Arnheim, and R. Sternglanz, 1973, J. Biol. Chem. **248**:7247–7252).

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