Effect of Bacteriophage Ghost Infection on Protein Synthesis in *Escherichia coli*

ISAMU FUKUMA AND AKIRA KAJI

Department of Microbiology, University of Pennsylvania School of Medicine,
Philadelphia, Pennsylvania 19104

Received for publication 5 June 1972

The rate of protein synthesis by *Escherichia coli* markedly decreased within 1 min after phage T4 infection, whereas a complete cessation of protein synthesis was observed within at least 25 sec after T4 ghost infection. The cellular level of amino acids and aminoacyl-transfer ribonucleic acid (tRNA) did not change drastically upon infection with ghosts, indicating that the inhibition of protein synthesis took place at a step(s) beyond aminoacyl-tRNA formation. The host messenger RNA remained intact and still bound to ribosomes shortly after ghost infection. Kinetic studies of the effect of ghosts on host protein synthesis revealed that nascent peptide chains on ribosomes were not released upon ghost infection.

The infection of *Escherichia coli* with T-even bacteriophage results in a rapid and large reduction of host-specific macromolecular synthesis and start of phage-specific macromolecular synthesis (2-5, 13, 15, 18, 20, 23). There have been a number of reports which suggest that infection results in modifications of components of the host protein synthesizing machinery, such as transfer ribonucleic acid (tRNA) (30, 31), aminoacyl-tRNA synthetase (21, 22), and initiation factor (6, 17, 26, 29). These modifications require protein synthesis in the infected bacterial cells. Unlike the infection with intact phage, infection with DNA-less phage particles, or "ghosts," results in a marked inhibition of cellular respiration (12), and synthesis of protein, deoxyribonucleic acid (DNA), and RNA are all inhibited more efficiently by ghosts than by intact phage (7, 8, 10, 12). However, the exact mechanism through which ghost infection inhibits host macromolecular synthesis remains obscure at the present moment.

In the experiments described in this communication, cells of *E. coli* B were infected with bacteriophage ghosts prepared by osmotic shock from phage T4D, and the effect of the ghost infection on the various steps involved in cellular protein synthesis was studied. It was found that *E. coli* protein synthesis was completely stopped within 25 sec after ghost infection. During this period, the level of amino acids, aminoacyl-tRNA, and messenger RNA (mRNA) appeared to stay the same as in the control cells. Data presented in this paper are consistent with the

hypothesis that movement of ribosomes on mRNA may suddenly stop after ghost infection.

MATERIALS AND METHODS

Organisms used. *E. coli* B was used as the host bacteria sensitive to phage T4D and its ghosts, and for the growth of T4D. An amber mutant of T4D, *amb22*, which has a mutation in gene 43 (DNA polymerase) and which does not express its late gene functions in the nonpermissive host (*E. coli* B), was grown on *E. coli* CR63.

Radioisotopes. L-¹⁴C-valine (248 mCi/mmmole) and ¹⁴C-uracil (30 mCi/mmmole) were obtained from New England Nuclear Corp. Radioactivity was determined by a Tri-Carb liquid scintillation spectrometer with an efficiency of 10⁶ counts per min per μ Ci.

Culture media. Tris medium was prepared according to Kaempfer and Magasanik (18) in a medium of the following composition: 0.12 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5), 0.08 M NaCl, 0.02 M KCl, 0.02 M NH₄Cl, 10⁻³ M MgSO₄, 3.5 $\times$ 10⁻⁴ M CaCl₂, 2 $\times$ 10⁻⁶ M FeCl₃, 2.5 $\times$ 10⁻³ M Na₂SO₄, and 10⁻⁴ M K₂HPO₄. TG medium is the Tris medium supplemented with glycerol to a final concentration of 0.5% by volume. TGA medium is the TG medium supplemented with vitamin-free Casamino Acids to a final concentration of 0.2%. TY broth contained 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter.

Growth and infection of bacteria. Cells of *E. coli* B were grown at 37 C in an Eberbach reciprocal shaker. Growth was measured in a Klett colorimeter, and the Klett reading had been calibrated to the number of colony formers in the culture media. The mass doubling times were 50, 50, and 30 min, in TG medium, TGA medium, and TY broth, respectively. Cells were grown to a density of 1 to 5 $\times$ 10⁸ cells/ml and were

infected with phage or ghosts in the presence of L-tryptophan (final concentration 10 to 20 $\mu\text{g/ml}$) at the multiplicity of infection (MOI) indicated in the figures and tables. Since most of the experiments were performed with MOI of 5 to 10, most of the cells had lost their viability by the infection.

Preparation and titration of phage T4 ghosts. Phage T4 was grown on *E. coli* B according to Adams (1). Ghost stocks were prepared by osmotic shock of bacteriophage T4 and T4 *amb22* by the method of Duckworth (*personal communication*). Two volumes of 3 M sodium acetate were added to one volume of the phage suspension which titered between 1×10^{12} to 1×10^{13} PFU/ml. Equilibration of the phage in the salt solution for 15 min at 0°C was followed by rapid dilution into 100 volumes of ice-cold distilled water. Inactivation of plaque-forming ability was usually greater than 97%. To this solution were added deoxyribonuclease (5 $\mu\text{g/ml}$ final concentration) and MgCl_2 (1 mM final concentration). This was incubated at 37°C for 15 min and centrifuged in a Sorvall SS-34 rotor at 6,000 rev/min for 15 min. The supernatant fluid was further centrifuged at $150,000 \times g$ for 2 hr in a Beckman Spinco ultracentrifuge. The pellets containing the ghosts were suspended in a small volume of the Tris medium and washed three times with the Tris medium. The final suspension was stored at 4°C until used. Approximately 50 to 60% of the original titer was recovered in this preparation.

The ghosts were titered by a modification of the method of Duckworth and Bessman (9). Several 10-ml cultures of *E. coli* B (2 to 4×10^8 cells/ml) in TGA medium, nutrient broth, or in TY broth were placed in 125-ml Erlenmeyer flasks and supplemented with 20 μg of L-tryptophan per ml. Known volumes of ghost preparation were added to the cultures and the mixtures were allowed to stand at room temperature for 2 min. To each of these was added 0.1 ml of 0.05 M isopropyl- β -D-thiogalactopyranoside, and the cultures were incubated with shaking at 37°C for 15 min. The cells were collected, washed once with Tris medium, and suspended in 5 ml of 0.02 M sodium phosphate buffer, pH 7.5. Each of the suspensions was treated with 0.2 ml of toluene at 37°C for 30 min with occasional vigorous stirring. Toluene was then removed by bubbling air through the suspension. Cell suspensions thus treated were assayed for β -galactosidase activity. One milliliter of the incubation mixture for the assay of β -galactosidase was made by mixing 0.2 ml of the suspension, 0.6 ml of 0.02 M sodium phosphate buffer (pH 7.5), and 0.2 ml of 0.005 M orthonitrophenyl- β -D-galactopyranoside. The rate of hydrolysis of the substrate was determined by measuring the increase in the absorbancy at 420 nm for 5 min at 25°C. The enzyme activity was expressed by increase of optical density at 420 nm per min per unit volume of the suspension. The decrease in inducibility of β -galactosidase (as percent of the uninfected control) and the volume of the ghost suspension added were plotted, and the ghost titer was calculated from the 37% survival point at which the number of ghosts is presumably equal to the number of bacteria in the culture.

RESULTS

Effect of phage and ghost infection on amino acid incorporation by *E. coli*. Figure 1 illustrates the effect of infection with phage or ghosts on the incorporation of ^{14}C -valine into protein by host cells. It is clear that infection with intact phage caused only a partial decrease in the rate of protein synthesis, whereas infection with ghosts caused an immediate and complete cessation. In both cases, the effect of infection was already evident 1 min after the addition of phage or ghosts.

To determine how rapidly the inhibition takes place upon ghost infection, the capacity of the infected cells to incorporate amino acids was tested in the following manner. The cells were exposed to ghosts for various periods, mixed with a culture medium containing ^{14}C -valine, and allowed to incorporate the amino acid into protein for 5 min. The results shown in Table 1 indicate that the addition of ghosts 15 sec prior to the addition of the radioactive amino acid reduced the incorporation in the subsequent 5 min to approximately 0.05% of the control. Thus, the amount of amino acids incorporated by the infected cells during 5 min corresponds to the amount of amino acids incorporated by uninfected cells during 0.15 sec.

To obtain precise information about the rapidity with which amino acids are incorporated into protein fractions by *E. coli* cells, a time course

FIG. 1. Effect of infection with phage or ghosts on the incorporation of ^{14}C -valine into protein by *E. coli* B. Cells were grown in TG medium to a density of 5×10^8 cells/ml. The culture (10 ml) was mixed with 2 μCi of ^{14}C -valine, 15 μg of ^{14}C -valine, and incubated at 37°C in a 50-ml flask for 8.5 min. At this point, T4 *amb22* (MOI of 7.5) or its ghosts (MOI of 5.8) were added to the culture. Samples (0.1 ml) were removed at the time indicated and the hot trichloroacetic acid-insoluble radioactivity was determined. Symbols: $\circ$ — $\circ$, uninfected cells; $\bullet$ — $\bullet$, cells infected with phage (A) or ghosts (B).

TABLE 1. Amino acid incorporation into protein by cells exposed to phage ghosts for various periods^a

Expt	Time of addition (sec)		Radioactivity incorporated (counts per min/1.0 ml/5 min)
	Ghost	¹⁴ C-Valine	
1		0	13,314
2	0	15	8
3	0	32	5
4	0	48	0

^a A culture (7 ml) of *E. coli* B (5.3×10^8 cells/ml) in TG medium was placed in a 50-ml flask and shaken at 37 C. In experiment 1, a 1.4-ml sample was removed, mixed immediately with 0.1 μ Ci of ¹⁴C-valine and 1.5 μ g of ¹²C-valine, and then incubated at 37 C for 5 min. In experiments 2 to 4, ghosts of T4 *amb22* were added to the culture at MOI of 8.5. Samples (1.4 ml) were removed at 15, 32, and 48 sec after the addition of ghosts and incubated with ¹⁴C- and ¹²C-valine as described above. Incorporation of the amino acid was terminated by the addition of 1.5 ml of 10% trichloroacetic acid 5 min after the addition of the radioactive amino acids.

experiment was carried out in which cells were exposed to ¹⁴C-valine for a short period, and the radioactivity incorporated into proteins was determined. As shown in Fig. 2, after the addition of the amino acid, approximately 10 sec were required before the observable onset of the incorporation of radioactivity into protein. These observations suggest that the effect of ghost infection on host protein synthesis was manifested within 25 sec after the infection. This rapid effect of ghost infection is consistent with the earlier observations that cells induced for β -galactosidase synthesis lose the capacity to form the enzyme immediately after infection with ghosts (7, 10).

Effect of ghost infection on precursors of protein synthesis. It has been established that the infection of *E. coli* with T-even bacteriophages makes the cell more permeable to certain acridine dyes (11, 16, 27), and causes leakage of certain cations (28) and ³²P-labeled materials (24, 25) from the infected cells. It therefore seemed possible that the sudden change in the rate of protein synthesis by *E. coli* after infection might be due to the loss of precursors of protein (amino acids and aminoacyl-tRNA). To examine this possibility, T4 ghosts were added to a culture of *E. coli* which had been actively incorporating ¹⁴C-valine for 2 min, and the radioactivity retained by these cells was determined. As shown in Fig. 3, the total cellular radioactivity decreased only slowly. To estimate the size of the precursor pool, a pulse-

chase experiment was performed. In this experiment, cells were given ¹⁴C-valine for 2 min and the ¹²C-valine was added. As can be seen in this figure, after the addition of the unlabeled amino acid, the total cellular radioactivity decreased rapidly and then reached a plateau. The extent of the decrease due to ghost infection during the first minute was approximately 50% of the decrease caused by the addition of ¹²C-valine. These results suggest that the sudden cessation of host protein synthesis by ghost infection may not be caused by depletion of amino acids from cells because cessation of host protein synthesis took place much earlier than the decrease of cellular radioactivity by ghost. However, the possibility still remains that the 50% decrease of free amino acid within 1 min after the infection with ghost may have some bearing on the sudden cessation of protein synthesis.

A possibility existed, however, that the intracellular level of aminoacyl-tRNA might have dropped suddenly after ghost infection. To examine this possibility, cells were labeled with ¹⁴C-valine, and the radioactivity associated with RNA was determined before and after infection with ghosts. As shown in Table 2, the radioactive amino acid associated with RNA, which is sensi-

FIG. 2. Time course of the incorporation of ¹⁴C-valine into protein by *E. coli* B. Cells were grown in TG medium to a density of 5.2×10^8 cells/ml. The culture (10 ml) was mixed with 1 μ Ci of ¹⁴C-valine and 15 μ g of ¹²C-valine and then incubated at 37 C. Samples (1.4 ml) were removed at various intervals and the hot trichloroacetic acid-insoluble radioactivity was determined.

FIG. 3. Effect of ghost infection on the size of the amino acid pool in cells. A culture (2 ml) of *E. coli* B (1.5×10^8 cells/ml in TG medium) was mixed with $0.2 \mu\text{Ci}$ of ^{14}C -valine and incubated at 37°C . Two minutes after the addition of ^{14}C -valine, T4 ghosts (MOI of 8) or $50 \mu\text{g}$ of ^{12}C -valine or both were added. Samples (0.05 ml) were removed at the various intervals indicated. The sample was immediately poured onto a Millipore filter (type HA; pore size $0.45 \mu\text{m}$) which had been washed once with the culture medium. The cells remaining on the filter paper were quickly washed once with 5 ml of cold (0 to 4°C) culture medium. The washing process took less than 10 sec. The membrane filters were dried, and the radioactivity was measured. Symbols: ●—●, infected with ghost; ○—○, ^{12}C -valine was added; △, infected with ghost and ^{12}C -valine was added. The arrow indicates the time when ghosts were added.

tive to either ribonuclease or an alkaline pH, did not change appreciably after ghost infection. These observations indicate that the ghost infection caused a slow leakage of free amino acids but did not cause the net deacylation or loss of aminoacyl-tRNA. It appears, therefore, that the adsorption of ghosts caused the inhibition of protein synthesis at a step beyond aminoacyl-tRNA formation. It should be pointed out, however, that data presented here merely show that the aminoacyl tRNA at the time of infection did not change the level upon infection. Although unlikely, the possibility still remains that ghost infection may influence the aminoacyl tRNA formation as well as other steps of protein synthesis.

Effect of ghost infection on cellular protein and RNA. The preceding experiments indicated that the amounts of aminoacyl-tRNA in cells did not decrease after infection with ghosts. Although soluble proteins required for protein synthesis

in vitro were also present at a nearly normal level in the infected cells (I. Fukuma and A. Kaji, *in preparation*), it was possible that the cessation of host protein synthesis was due to partial degradation of some of the proteins which may play an important role in protein synthesis in vivo. To examine this possibility, cells were labeled with ^{14}C -valine or ^{14}C -uracil by allowing growth for one generation, and the effects of ghost infection on labeled macromolecules were then studied. As shown in Table 3, there was virtually no leakage or degradation of proteins

TABLE 2. Effect of ghost infection on the size of the aminoacyl-tRNA pool in cells^a

Time of addition (sec)			Radioactivity associated with RNA (counts/min·0.2 ml)				
Expt	Ghost	CN ⁻	A No treatment	B After treatment with ribonuclease	C After incubation at pH 9	Decrease	
						A-B	A-C
1		50	263	49	85	214	178
2	73	90	258	18	84	240	174
3		120	259	11	92	248	167
4	73	150	266	16	92	250	174

^a Cells were grown in TG medium to a density of 2×10^8 cells/ml. The culture (10 ml) was mixed with $5 \mu\text{Ci}$ of ^{14}C -valine at zero time and incubated at 37°C . In experiments 2 and 4, ghosts were added at an MOI of 8. To stop metabolic processes, potassium cyanide (0.001 M final concentration) was added to the culture at the time indicated. Cells were collected by centrifugation and suspended in a buffer containing 5 mM MgCl_2 , 0.06 M KCl, and 0.05 M sodium acetate (pH 5.5). After the suspension was frozen in dry ice-acetone and thawed at 37°C three times, 0.1 ml of 5% sodium dodecyl sulfate and 2.1 ml of phenol saturated with the above mentioned buffer were added and the mixture was shaken for 5 min at room temperature. To the aqueous layer (1.8 ml) were added 1 mg of tRNA as a carrier, 0.2 ml of 20% sodium acetate (pH 5.2), and 4 ml of cold ethanol. The resulting mixture was allowed to stand at -20°C overnight. The precipitate was dissolved in 2 ml of 0.01 M potassium acetate buffer (pH 5.6). A portion (0.2 ml) of the sample was mixed with 0.8 ml of the same buffer containing $25 \mu\text{g}$ of ribonuclease per ml (Worthington Biochemical) and the mixture was incubated at 30°C for 15 min (b). Another portion (0.2 ml) was mixed with 0.8 ml of 0.1 M Tris-hydrochloride buffer (pH 9.0), and the mixture was incubated at 30°C for 60 min (c). After these treatments, RNA was precipitated with cold (4°C) 5% trichloroacetic acid, collected on a Millipore filter, and the radioactivity was counted.

TABLE 3. Effect of ghost infection on cellular protein and RNA^a

Expt	Label	Acid-insoluble radioactivity (counts per min per ml)			
		Cells		Supernatant	
		Before infection	After infection	Before infection	After infection
1	¹⁴ C-uracil	6162	6333	220	198
2	¹⁴ C-valine	7039	7074	141	145

^a Expt. 1: A culture of *E. coli* B (20 ml, 2×10^8 cells/ml in TG medium) was incubated with 2 μ Ci of ¹⁴C-uracil at 37 C for 5 min, washed once with the medium, and again incubated in 20 ml of fresh TG medium for 1 hr. Portions (3 ml) of the culture were removed before and 2 min after infection with T4 ghosts (MOI of 8). The cold trichloroacetic acid-insoluble radioactivity of 1 ml of the sample was determined. The rest of the sample (2 ml) was centrifuged at $5,000 \times g$ for 10 min and the cold trichloroacetic acid-insoluble radioactivity of 1 ml of the supernatant fluid was also determined. Expt. 2: Procedures were identical with those described above except that cells were labeled with ¹⁴C-valine and hot (95 C) trichloroacetic acid-insoluble radioactivity was determined.

and RNA into acid-soluble materials within 2 min after ghost infection.

Effect of ghost infection on host mRNA. To examine the possibility that the cessation of host protein synthesis may be caused by a sudden degradation of host mRNA, the experiment shown in Fig. 4 was carried out. In this experiment, a culture of *E. coli* B was labeled with ¹⁴C-uracil during a short period and infected with T4 ghosts. The RNA extracted from this culture was then analyzed by sucrose density gradient centrifugation. It can be seen in this figure that the distribution of radioactivity on the gradient was not influenced by infection with ghosts. From this result it can be concluded that degradation of mRNA molecules present at the time of infection did not occur within the short period after ghost infection.

A possibility existed, however, that the cessation of host protein synthesis might be caused by a rapid release of host mRNA from ribosomes. To explore this possibility, the experiments shown in Fig. 5 were performed. In this experiment, a culture of cells was labeled with ¹⁴C-uracil for a short period, infected with ghosts, and ribosomes were isolated. As shown in Fig. 5a, the addition of ghosts to the culture inhibited the incorporation of ¹⁴C-uracil into RNA. However,

the RNA synthesized prior to ghost infection remained associated with ribosomes even after ghost infection (Fig. 5b). A remote possibility that some of the labeled RNA was integrated into the ribosomal structure in the form of ribosomal RNA was ruled out by a separate observation that the radioactivity on ribosomes could be released by treating a ribosomal complex with a buffer containing a low concentration of magnesium (10^{-4} M). These observations suggest that the infection with ghosts has no appreciable influence on preexisting host mRNA.

Fate of nascent polypeptides on ribosomes after ghost infection. One can imagine that the sudden

FIG. 4. Effect of ghost infection on the size of cellular mRNA. Cells were grown in TGA medium to a density of 4×10^8 cells/ml. The culture (20 ml) was mixed with 2 μ Ci of ¹⁴C-uracil and incubated at 37 C for 32 sec. At this point, 10 ml of the culture was chilled by pouring into 20 ml of cold (-20 C) ethanol and, at the same time, T4 ghosts were given to the rest (10 ml) of the culture (MOI of 8). The infected culture was incubated for an additional 24 sec at 37 C and then chilled in the same manner. The precipitated cells were collected by centrifugation, and RNA was extracted with phenol. The RNA was dissolved in 1.5 ml of 0.01 M Tris-hydrochloride (pH 7.5). A portion (0.3 ml) of this solution was placed on top of a 5-ml sucrose gradient (5 to 20%) containing 0.005 M EDTA, 0.05 M NaCl, and 0.01 M sodium acetate (pH 5.1). Tubes were centrifuged in a Beckman Spinco rotor SW50.1 at 50,000 rev/min for 210 min. Two-drop fractions were collected from the bottom of the tube. Cold trichloroacetic acid-insoluble radioactivity of each fraction was determined. A mixture of ribosomal RNA (16S and 23S rRNA) was sedimented under the identical conditions and positions of these rRNA were monitored by absorption at 260 nm (...).

FIG. 5. Effect of ghost infection on the interaction of mRNA with ribosomes. Cells of *E. coli* B were grown in TGA medium to a density of 4×10^8 cells/ml. The culture (70 ml) was mixed with 7 μ Ci of ¹⁴C-uracil and incubated at 37 C. Ghosts of T4 were given to 35 ml of the culture at 47 sec after the addition of ¹⁴C-uracil (MOI of 8). Samples (5 ml) of the culture were removed at various time intervals and immediately mixed with 5 μ liters of 1 M potassium cyanide and nonradioactive carrier *E. coli* B (5×10^8 cells) in a centrifuge tube. Cells were collected, suspended in 2 ml of buffer containing 0.001 M Tris-hydrochloride (pH 7.8), 0.01 M Mg acetate, 0.06 M KCl, and 0.006 M 2-mercaptoethanol, sonically treated, and ribosomes were isolated by a differential centrifugation. Values in graphs represent (a) the cold trichloroacetic acid-insoluble radioactivity of whole cell and (b) S-30 (supernatant of the sonic extract of the cells after centrifugation at $30,000 \times g$ for 30 min) and ribosomal fractions from 0.05 ml of the original culture. A similar experiment, in which the carrier cells were not added during the preparation of the extracts, showed that the ribosome-associated radioactivity did not change within 24 sec after ghost infection. a, Whole cell; $\circ$, uninfected; $\bullet$, infected. b, Extract; $\circ$, total radioactive RNA in S-30 of control cells; $\bullet$, total radioactive RNA in S-30 of infected cells; $\triangle$, ribosomal radioactivity of control cells; $\blacktriangle$, ribosomal radioactivity of infected cells.

stopping of host protein synthesis is taking place at a specific step of protein synthesis, such as chain initiation, chain elongation, or chain termination. It is also possible that an abnormal release of nascent peptide molecules from ribosomes may be responsible for the sudden cessation of protein synthesis.

In an attempt to pinpoint the action of ghosts among these possibilities, the experiment shown in Fig. 6 was performed. In this experiment, ¹⁴C-valine was added to a culture of *E. coli*, and ghosts were added 18 sec after the addition of the labeled amino acid. The incorporation of ¹⁴C-valine into total protein, the soluble fraction, and the ribosomal fraction was determined at various times before and after infection. As can be seen in Fig. 6a, infection with ghosts did not significantly decrease the radioactivity associated with ribosomes, but it considerably inhibited the increase of the radioactivity in the soluble fraction during this period. Figure 6b shows the time course of the ratio of radioactivity on ribosomes to the radioactivity of total proteins. This ratio rapidly decreased in uninfected control cells and approached an asymptotic level about 60 sec after the addition of ¹⁴C-valine. However, infection with ghosts virtually stopped the decrease in this ratio, and the ratio at the moment of infection remained almost unchanged thereafter. These results ruled out the possibility that the infection might have caused an abnormal release of nascent peptide chains from ribosomes.

DISCUSSION

The data presented in this paper indicate that the incorporation of amino acids into protein by *E. coli* was completely inhibited within 25 sec after infection with ghosts. The infection with intact phage, on the other hand, caused a decrease (about 50% in the rate of incorporation within 1 min following infection. Kaempfer and Magasanik (18) observed that preinduced *E. coli* cells could continue to synthesize β -galactosidase for about 3 min after T-even infection at about 50% of the rate observed in control cells. The synthesis of the enzyme, however, was completely blocked upon infection with T4 ghosts (10). These observations suggest that ghost-infected cells are totally inert for protein synthesis, whereas infection with phage only partially inhibits cellular protein synthesis. In addition, the inhibitory effect of ghost infection is more rapid than the effect of intact phage infection. The amount of cellular valyl-tRNA did not change upon ghost infection, suggesting that the inhibition of cellular protein synthesis by ghosts probably takes place at the step(s) beyond the formation of aminoacyl-tRNA. The observed retention

FIG. 6. Effect of ghost infection on the growth of nascent peptide chains in host cells. A culture of *E. coli* B (50 ml, 1.6×10^8 cells/ml in TG medium) was mixed with 5 μCi of ^{14}C -valine, and incubated at 37 C. T4 ghosts were given to 25 ml of the culture (MOI of 8) at 18 sec after the addition of ^{14}C -valine. Samples (5 ml) were removed at various time intervals and poured into a cold (0 C) centrifuge tube containing 0.04 ml of 1 M KCN and 1.5 ml of a nonradioactive culture of *E. coli* B (7×10^8 cells). Cells were collected by centrifugation, sonically treated in 2 ml of buffer, containing 0.01 M Tris-hydrochloride (pH 7.4), 0.01 M MgSO_4 , 0.05 M NH_4Cl , and 0.006 M 2-mercaptoethanol, and fractionated by a differential centrifugation. Hot trichloroacetic acid-insoluble radioactivities of the whole extract, soluble fraction, and ribosomal fraction were determined. The radioactivities incorporated into the soluble fraction (abbreviation: Sup, supernatant after centrifugation at $155,000 \times g$ for 1 hr), and the ribosomal fraction (a), and the ratios of radioactivity between the ribosomal fraction and the whole extract (b), are plotted against the time after the addition of ^{14}C -valine. Symbols: $\circ$ and Δ , uninfected cells; $\bullet$ and $\blacktriangle$ infected cells; Sup (U), soluble fraction from uninfected cells; Sup (I), soluble fraction from infected cells.

of aminoacyl-tRNA by the ghost-infected cells is in agreement with the observation that there was very little leakage of RNA from the infected cells.

The incorporation of uracil into RNA by *E. coli* was stopped by ghost infection as quickly as was the incorporation of amino acids into protein, but the sedimentation behavior of pre-existing mRNA did not change for as long as 24 sec after infection with ghosts. In addition, the amount of pulse-labeled RNA bound to ribosomes did not change after infection with ghosts. It therefore appears probable that most of the preexisting polyribosomes are still present at 24 sec after ghost infection. This suggests that cellular protein synthesis comes to an immediate halt upon ghost infection, leaving the polysomal structure intact. In a similar fashion, Hattman and Hofschneider (14) found that in simultaneous mixed infection with T4 *am55* and RNA phage M12, the parental RNA forms polysomes as efficiently as in control without T4 infection.

In an attempt to study the action of ghosts in more detail, cells incorporating ^{14}C -valine into protein for a very short period were infected with ghosts, and the time course of the change in the radioactivity associated with

ribosomes and the ratio of radioactivity of ribosomes to the radioactivity of total protein were studied. This ratio remained unchanged after ghost infection. These data are consistent with a hypothesis that ribosomal movement on mRNA is rapidly halted upon ghost infection. This conclusion agrees with the observation that mRNA is still intact and bound to ribosomes after infection. It appears, therefore, that a sudden release of nascent peptides from ribosomes does not take place after ghost infection.

LITERATURE CITED

- Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
- Benzer, S. 1953. Induced synthesis of enzymes in bacteria analyzed at the cellular level. *Biochim. Biophys. Acta* 11:383-395.
- Burton, K. 1955. The relation between the synthesis of deoxyribonucleic acid and the synthesis of protein in the multiplication of bacteriophage T2. *Biochem. J.* 61:473-483.
- Cohen, S. S. 1948. The synthesis of bacterial viruses. I. The synthesis of nucleic acid and protein in *E. coli* B infected with T2r⁺ bacteriophage. *J. Biol. Chem.* 174:281-303.
- Cohen, S. S., and T. F. Anderson. 1946. Chemical studies on host-virus interactions. I. The effect of bacteriophage adsorption on the multiplication of its host, *Escherichia coli* B. *J. Exp. Med.* 84:511-523.
- Dube, S. K., and P. S. Rudland. 1970. Control of translation by T4 phage: altered binding of disfavoured messengers. *Nature (London)* 226:820-823.

7. Duckworth, D. H. 1970. The metabolism of T4 phage ghost infected cells. I. Macromolecular syntheses and transport of nucleic acid and protein precursors. *Virology* 40:673-684.
8. Duckworth, D. H. 1970. Biological activity of bacteriophage ghosts and "take-over" of host functions by bacteriophage. *Bacteriol. Rev.* 34:344-363.
9. Duckworth, D. H., and M. J. Bessman. 1965. Assay for the killing properties of T2 bacteriophage and their "ghosts". *J. Bacteriol.* 90:724-728.
10. Fabricant, R., and D. Kennell. 1970. Inhibition of host protein synthesis during infection of *Escherichia coli* by bacteriophage T4. III. Inhibition by ghosts. *J. Virol.* 6:772-781.
11. Foster, R. A. C. 1948. An analysis of the action of proflavine on bacteriophage growth. *J. Bacteriol.* 56:795-809.
12. French, R. C., and L. Siminovitch. 1955. The action of T4 bacteriophage ghosts on *E. coli* B. *Can. J. Microbiol.* 1:757-774.
13. Hall, B. D., and S. Spiegelman. 1961. Sequence complementarity of T2-DNA and T2-specific RNA. *Proc. Nat. Acad. Sci. U.S.A.* 47:137-146.
14. Hattman, S., and P. H. Hofschneider. 1968. Influence of T4 on the formation of RNA-phage specific polyribosomes and polymerase. *J. Mol. Biol.* 35:513-522.
15. Hershey, A. D., J. Dixon, and M. Chase. 1953. Nucleic acid economy in bacteria infected with bacteriophage T2. I. Purine and pyrimidine composition. *J. Gen. Physiol.* 36:777-789.
16. Hessler, A. Y. 1965. Acridine resistance in bacteriophage T2H as a function of dye penetration measured by mutagenesis and photoinactivation. *Genetics* 52:711-722.
17. Hsu, W. T., and S. B. Weiss. 1969. Selective translation of T4 template RNA by ribosomes from T4-infected *Escherichia coli*. *Proc. Nat. Acad. Sci. U.S.A.* 64:345-351.
18. Kaempfer, R. O. R., and B. Magasanik. 1967. Effect of infection with T-even phage on the inducible synthesis of β -galactosidase in *E. coli*. *J. Mol. Biol.* 27:453-468.
19. Kennell, D. 1970. Inhibition of host protein synthesis during infection of *Escherichia coli* by bacteriophage T4. II. Induction of host messenger ribonucleic acid and its exclusion from polysomes. *J. Virol.* 6:208-217.
20. Levine, A. P., and K. Burton. 1961. Inhibition of enzyme formation following infection of *E. coli* with phage T2r⁺. *J. Gen. Microbiol.* 25:307-314.
21. Neidhardt, F. C. 1966. Roles of amino acid activating enzymes in cellular physiology. *Bacteriol. Rev.* 30:701-719.
22. Neidhardt, F. C., and C. F. Earhart. 1966. Phage-induced appearance of a valyl sRNA synthetase activity in *Escherichia coli*. *Cold Spring Harbor Symp. Quant. Biol.* 31:557-563.
23. Nomura, M., C. Witten, N. Mantei, and H. Echols. 1966. Inhibition of host nucleic acid synthesis by bacteriophage T4: effect of chloramphenicol at various multiplicities of infection. *J. Mol. Biol.* 17:273-278.
24. Puck, T. T., and H. H. Lee. 1954. Mechanism of cell wall penetration by viruses. I. An increase in host cell permeability induced by bacteriophage infection. *J. Exp. Med.* 99:481-494.
25. Puck, T. T., and H. H. Lee. 1955. Mechanism of cell wall penetration by viruses. II. Demonstration of cyclic permeability change accompanying virus infection of *E. coli* B cells. *J. Exp. Med.* 101:151-175.
26. Schedl, P. D., R. E. Singer, and T. W. Conway. 1970. A factor required for the translation of bacteriophage f2 RNA in extracts of T4-infected cells. *Biochem. Biophys. Res. Commun.* 38:631-637.
27. Silver, S. 1965. Acriflavine resistance: a bacteriophage mutation affecting the uptake of dye by the infected bacterial cells. *Proc. Nat. Acad. Sci. U.S.A.* 53:24-30.
28. Silver, S., E. Levine, and P. M. Spielman. 1968. Cation fluxes and permeability changes accompanying bacteriophage infection of *Escherichia coli*. *J. Virol.* 2:763-771.
29. Steitz, J. A., S. K. Dube, and P. S. Rudland. 1970. Control of translation by T4 phage: altered ribosome binding at R17 initiation sites. *Nature (London)* 226:824-827.
30. Sueoka, N., and T. Kano-Sueoka. 1964. A specific modification of leucyl-sRNA of *E. coli* after phage T2 infection. *Proc. Nat. Acad. Sci. U.S.A.* 52:1535-1540.
31. Waters, L. C., and G. D. Novelli. 1967. A new change in leucine transfer RNA observed in *Escherichia coli* infected with bacteriophage T2. *Proc. Nat. Acad. Sci. U.S.A.* 57:979-985.