Alterations in the Distribution of Labeled Ribonucleic Acid in Polyribosomes from *Escherichia coli* Infected with Bacteriophage R17

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The distribution of labeled ribonucleic acid (RNA) associated with polysomes from *Escherichia coli* infected with the bacteriophage R17 was investigated. Pulse-labeling of RNA for 15 sec with ^3H -uridine resulted in increased labeling of the RNA associated with larger polysomes from infected cells as compared to control cells. Analysis of the RNA indicated that the increased labeling of large polysomes resulted from the presence of labeled double-stranded viral RNA. Other species of 15-sec pulse-labeled RNA entered into polysome formation in both infected and control cells. On the other hand, pulse-labeling of cultures for 15 sec with ^3H -uridine followed by a 5-min chase with unlabeled uridine resulted in a greater decrease in the amount of labeled RNA associated with large polysomes from infected cells as compared to control cells. This decreased labeling of large polysomes from infected cells was accompanied by an increased amount of label associated with the monomer to trimer regions. Analysis of RNA labeled under pulse-chase conditions indicated that virus infection resulted in an increased amount of heterogeneous 5 to 15S RNA in both the monomer to trimer and ribosomal subunit-soluble regions of the polysome profile. Labeled 5 to 15S RNA extracted directly from infected cells under pulse-chase conditions, without prior polysome fractionation, was characterized by a shift toward a distribution of smaller polynucleotides.

Infection of *Escherichia coli* with the bacteriophage R17 results in an altered polyribosome distribution characterized by a decrease in the number of large polysomes and an increase in the number of smaller polysomes (5, 6). At approximately the same time during virus infection, cellular ribonucleic acid (RNA) synthesis (2; Iglewski, *submitted for publication*) and protein synthesis (2,4) are inhibited. The inhibition of host RNA synthesis appears to be the result of a specific inhibition of ribosomal RNA (rRNA), because the incorporation of labeled precursors into other types of cellular RNA is not altered (2, 8; Iglewski, *submitted for publication*). The depression of rRNA synthesis results in decreased ribosome biosynthesis (7). These alterations in the macromolecular synthesis of infected cells are accompanied by a shift toward viral-specific synthesis. Sugiyama and Stone (18) demonstrated, in MS2 phage-infected cells, a shift from synthesis of cellular proteins to synthesis of viral proteins. The alteration in the type of proteins synthesized dur-

ing infection suggests that a high degree of specificity is involved in the mechanism of inhibition of protein synthesis.

Hotham-Iglewski and Franklin (5) demonstrated that the absorbance profile of polysomes obtained from R17 phage-infected cells progressively shifts toward smaller polysomes as infection proceeds. This alteration in the polysome distribution suggests that the overall depression of protein synthesis in infected cells may result from an inhibition at the level of translation of messenger RNA (mRNA). The mechanism involved in this inhibition is unknown. An inhibition at the translational level, characterized by an accumulation of smaller polysomes, may involve the hindrance of movement of ribosomes along the message after formation of the initiation complex as suggested by Phillips et al. (14), nuclease activation causing a degradation of polysomes, or the disaggregation of polysomes.

To clarify the mechanism involved in this shift to smaller polysomes in R17-infected cells, the

distribution of labeled RNA associated with polysomes was investigated. The data indicate that 15-sec pulse-labeled RNA enters into polysome formation in both infected and control cultures. However, during pulse-chase experiments, the labeled RNA from larger polysomes of infected cells is relocated in smaller polysomes. This shift in the labeled RNA distribution is accompanied by an increased amount of heterogeneous 5 to 15S RNA in both the monomer to trimer and the ribosomal subunit-soluble regions of the polysome profile. The distribution of labeled 5 to 15S RNA extracted directly from infected cells is characterized by a shift toward smaller sizes.

MATERIALS AND METHODS

Bacteria and bacteriophage. Cultures of *E. coli* strain 3000 were grown in TCG1 media as previously described (Iglewski, submitted for publication). Cells were harvested by centrifugation, resuspended in a small volume of TCG1, and infected with the R17 phage at a multiplicity of 10 plaque-forming units per cell (Iglewski, submitted for publication). The infected cells were resuspended in fresh, prewarmed TCG1 medium at a concentration of 4×10^8 cells/ml and incubated in a shaking water bath at 37 C.

Preparation of polyribosomes. Polyribosomes were extracted from cultures of *E. coli* by the method of Hotham-Iglewski and Franklin (5). The original procedure was slightly altered by using TMNa buffer instead of TMK buffer (12). Fifteen optical density units (at 260 nm) of the extracted polysomes were layered in a linear sucrose gradient (40 to 10% sucrose in TMNa buffer) and centrifuged in an SW41 rotor (Beckman Instruments, Inc.) at 40,000 rev/min for 70 min at 2 C. Optical density patterns were obtained by continuous monitoring of the sample at 260 nm with a Beckman spectrophotometer containing a flow-through cell. Fractions from labeled polysome preparations were collected at 10-sec intervals.

Preparation of labeled RNA. Cells were grown in TCG1 medium and infected with R17 as described. At 45 min after infection, an infected culture and a control culture were pulse-labeled with uridine-5-³H of specific activity 28 Ci/mmol (Schwarz BioResearch, Orangeburg, N.Y.) for either 15 or 90 sec. The incorporation of label was stopped by pouring the culture over one-half volume of frozen, crushed TCG1 medium containing 5 mM sodium azide, 100 µg of chloramphenicol/ml, and 10 mM potassium cyanide. The labeling period was terminated more efficiently when cyanide, as well as rapid cooling, was used to harvest the culture. In pulse-chase experiments, cultures were pulse-labeled for 15 sec with ³H-uridine and then chased by adding a 100-fold excess of unlabeled uridine 5 or 10 min before harvesting the cells. The cells were removed from the TCG1 medium by centrifugation at 5 C, and either the total labeled RNA was extracted with cold phenol (3) or polyribosomes were extracted.

Polyribosomes, labeled with ³H-uridine, were analyzed by sedimentation in a 40 to 10% sucrose

gradient. A 25-µliter portion of each fraction was counted in a liquid scintillation counter to establish a profile of the labeled RNA. The remaining labeled RNA fractions were divided into three pooled fractions: (i) RNA associated with polysomes larger than trimers, (ii) RNA in the monomer to trimer region of the gradient, and (iii) RNA associated with the ribosomal subunit and soluble regions of the gradient. The fractions were incubated with 2% sodium dodecyl sulfate at 37 C for 15 min. After the RNA was precipitated with two volumes of ethanol at -20 C, the samples were redissolved in a sample buffer for polyacrylamide gel electrophoresis by the procedure of Pons and Hirst (15).

RESULTS

Alteration in the polysome distribution of infected cells. The polyribosome distribution of cells infected for 45 min with R17 was characterized by a decrease in large polysomes and in an increase in smaller polysomes (Fig. 1). The relative distribution of the various sizes of polysomes within the overall polysome profile was measured by planimetry of the tracing of the absorbance at 260 nm. There was a 46% decrease in absorbance of polysomes in the region of the profile representing four or more ribosomes per mRNA and a 45% increase in absorbance in the monomer in trimer region of polysomes from infected cells, as compared to control cells.

Distribution of labeled RNA associated with polysomes. The alteration in the polyribosome distribution of infected cells was examined by extracting polysomes from cells pulse-labeled with ³H-uridine for either 15 or 90 sec (Fig. 2a and b). The type of labeled RNA profile associated with polysomes was dependent on the length of time the cells were exposed to the labeled uridine. A uniform distribution of label among polysomes was observed when infected cells were pulse-labeled for 15 sec. Labeling of control cells under identical conditions resulted in a greater quantity of ³H-uridine-labeled RNA associated with smaller polysomes, as compared to larger polysomes. Sucrose gradient analysis of labeled RNA from polysome extracts of infected and control cultures resulted in 1.5 times the amount of radioactivity sedimenting in the region of the larger polysomes from infected cells, as compared to control cells (Table 1).

As the labeling time was increased to 90 sec, a shift occurred in the profile of labeled RNA associated with polysomes from infected and control cultures (Fig. 2b). The profile of radioactivity from infected cultures and control cultures labeled for a longer period of time was similar to the absorbance profile of the polysomes. More labeled RNA was associated with the smaller polysomes than larger polysomes of infected cells. Profiles

FIG. 1. Distribution of polysomes from uninfected cultures and R17 phage-infected cultures of *Escherichia coli*. Polysome extracts were prepared from cells 45 min after infection. Fifteen optical density units at 260 nm were layered on a 10 to 40% sucrose gradient and centrifuged at 40,000 rev/min in a Beckman SW41 rotor for 70 min at 2 C. The absorbance at 260 nm was continuously monitored with a flow-through cell mounted in a Beckman spectrophotometer. Numbers 1, 2, and 3 indicate the position of the polysomal monomers, dimers, and trimers, respectively.

from control cultures displayed a uniform labeling of RNA throughout the polyribosome distribution.

Characterization of pulse-labeled RNA extracted from polysomes. Polyacrylamide gel electrophoresis was used to determine the incorporation of labeled uridine into the various types of RNA associated with the labeled polyribosomes. Polyribosomes from control cultures and virus-infected cultures pulse-labeled for 15 sec with ^3H -uridine were selected for analysis. Labeled RNA fractions from a polyribosome distribution obtained by sedimentation in a sucrose gradient were pooled into three fractions (Fig. 2a and b). The fractions were treated with SDS, alcohol was precipitated, and the fractions were suspended in sample buffer for analysis by polyacrylamide gel electrophoresis. The profile of labeled RNA obtained from the polyacrylamide gel (Fig. 3a and b) was divided into eight regions representing: (1) 4S RNA, (2) small mRNA, (3) large mRNA, (4) 16S rRNA, (5) 18 to 21S RNA, (6) 23S rRNA, (7) very large RNA, and (8) viral RNA. The results obtained by polyacrylamide gel analysis of labeled RNA from the polyribosomes of virus-infected cultures and control cultures (Table 2) show that virus infection did not alter the amount of 15-sec pulse-labeled 4S RNA, mRNA, or 16S rRNA associated with polyribosomes. However,

FIG. 2. Distribution of labeled RNA associated with polysomes from uninfected cultures and R17 phage-infected cultures. Lysates were prepared from cultures 45 min after infection. Labeled lysate (2.8×10^6 counts/min) was layered on a 10 to 40% sucrose gradient and centrifuged at 40,000 rev/min for 70 min at 2 C. The ratio of the counts per minute per optical density unit (at 260 nm) from the lysate of the control culture to the infected culture was 1.29 when pulse-labeled for 15 sec and 1.57 when pulse-labeled for 90 sec. Symbols indicate the distribution of labeled RNA from uninfected cells (●---●) and R17 phage-infected cells (●—●) pulse-labeled (a) for 15 sec and (b) for 90 sec with ^3H -uridine. Numbers 1, 2, and 3 indicate the position of the polysomal monomers, dimers, and trimers, respectively. Roman numerals I, II, and III indicate pooled fractions from polysomes representing the tetramer and larger polysomes, monomer to trimer, and ribosomal subunit-soluble regions, respectively. The pooled fractions were used for subsequent studies.

there was less 16S rRNA in the ribosomal subunit-soluble region of the polysome profile from infected cells. An increased amount of labeled uridine appeared to be associated with the 18S to 21S region from the infected cells. This region of

TABLE 1. Distribution of ^3H -uridine-labeled RNA in polysome profiles from cells infected for 45 min with bacteriophage R17

Polysome fraction	Radioactivity in polysomes (%)			
	15-Sec pulse label		15-Sec pulse label preceding 5-min chase	
	Uninfected cells	Virus-infected cells	Uninfected cells	Virus-infected cells
I (>3 ribosomes/mRNA)	4.9	7.1	17.9	10.6
II (1-3 ribosomes/mRNA)	5.4	5.9	10.9	35.8
III (<70S)	89.7	87.0	69.2	53.6

the RNA profile represents the trough between the 16S and 23S rRNA bands. Quantitation of the amount of labeled RNA in this area was variable, and differences between infected cultures and control cultures were not considered significant. An increased amount of label was associated with the 23S rRNA from the large polysomes of infected cells. This increase may have resulted from the presence of labeled 27S single-stranded viral RNA that would not be well resolved from the 23S rRNA under the conditions employed for electrophoresis (Iglewski, *submitted for publication*). The large amount of labeled 23S rRNA in the monomer to trimer region of the polysomes from control cells resulted from the inclusion of a part of the 50S ribosomal subunit band during polysome fractionation. The 50S ribosomal subunits sedimented very near the monomer region of the polysomes in the sucrose gradient. The partial inclusion of the 50S ribosomal subunits in the monomer to trimer fraction also accounted for the decreased amount of labeled 23S rRNA in the ribosomal subunit region of the polysomes from the control cultures. Most of the labeled viral RNA intermediates are associated with large polysomes. Incorporation of ^3H -uridine into the viral RNA intermediates during the 15-sec pulse apparently was responsible for the increased amount of label found associated with the large polyribosome region from the virus-infected cells (Fig. 2a).

Distribution of ^3H -uridine-labeled RNA in polysomes after chase with unlabeled uridine. Pulse-chase experiments were used to determine the fate of the labeled RNA associated with polyribosomes from infected cells. Infected cultures and control cultures were pulse-labeled for 15 sec with ^3H -uridine and then chased with a 100-fold excess of unlabeled uridine for 5 min. Chasing with unlabeled uridine resulted in a more rapid decrease

FIG. 3. Polyacrylamide gel electrophoresis profile of labeled RNA from large polysomes of cells pulse-labeled for 15 sec with ^3H -uridine at 45 min after infection. Uninfected cultures and R17 phage-infected cultures were pulse-labeled with ^3H -uridine 15 sec before lysates were prepared. Polysomes were sedimented in a 10 to 40% sucrose gradient, and the gradient fractions were pooled into the three fractions indicated on the curves of Fig. 2. The labeled RNA from the various regions of the polysome gradients was analyzed by electrophoresis on 8.5-cm polyacrylamide gels containing 2.2% acrylamide and 1% agarose. The labeled RNA was further divided into eight classes of RNA as described in the text. A standard RNA preparation containing 4, 16, and 23S *E. coli* RNA and viral RF and RI was used as migration markers in the polyacrylamide gels. Distribution of labeled RNA from polysomes containing four or more ribosomes per messenger RNA from (a) R17 phage-infected cells and (b) uninfected cells.

in the labeled RNA content of the large polysomes from infected cultures, as compared to control cultures (Fig. 4). The decreased labeling of large polysomes in infected cells was accompanied by an increased amount of labeled RNA associated with the monomer to trimer region of the poly-

TABLE 2. *Distribution of RNA in polysomes from cells pulse-labeled for 15 sec with ^3H -uridine at 45 min after infection^a*

RNA fraction	Labeled RNA associated with polysomes (%)					
	I ^b (>3 ribosomes/mRNA)		II ^b (1-3 ribosomes/mRNA)		III ^b (<70S)	
	Uninfected cells	Virus-infected cells	Uninfected cells	Virus-infected cells	Uninfected cells	Virus-infected cells
4S RNA	5.4	5.0	9.0	7.6	43.0	43.6
Small mRNA	16.9	13.8	26.4	28.1	21.3	18.5
Large mRNA	34.3	36.5	23.4	28.6	14.7	13.3
16S rRNA	17.2	17.9	11.0	14.6	13.3	7.2
18-21S RNA	6.0	11.4	3.7	8.3	2.4	3.5
23S rRNA	16.4	23.8	25.0	15.9	3.4	7.3
Very large RNA	3.8	6.6	1.5	1.5	1.9	1.7
Viral RNA		36.0		4.6		1.9
Totals	100.0	151.0	100.0	109.2	100.0	97.0

^a Because the amount of labeled RNA associated with a polysome region differed between infected and control cultures, the percentage of label incorporated into each RNA component from the polyacrylamide gel was multiplied by the following correction factors, derived from the values given in Table 1, under "15-Sec pulse-label": region I, 7.1/4.9; region II, 5.9/5.4; region III, 87.0/89.7.

^b Roman numerals represent polysome regions shown in Fig. 2a.

somes. Nearly 50% of the labeled RNA found in the monomer to trimer region sedimented slightly faster than the 70S monomers. This labeled RNA probably represented single-stranded viral RNA encapsulated within mature phage particles.

Characterization of labeled RNA from polysomes after chase with unlabeled uridine. Polysomes from cells pulse-labeled for 15 sec with ^3H -uridine and then chased for 5 min with unlabeled uridine were divided into three fractions as previously described. The labeled RNA from each fraction was analyzed by polyacrylamide gel electrophoresis (Fig. 5a, b, and c). A decrease in all species of labeled cellular RNA was observed in the large polysomes from infected cells, as compared to control cells (Table 3). Most of the labeled mRNA of infected cells was found in the monomer to trimer region and in the ribosomal subunit-soluble regions of the polyribosome profile. Polyacrylamide gel electrophoresis of the RNA from the monomer to trimer fraction indicated that the vast majority of labeled RNA migrated in the 16 to 27S region of the gel. Of the labeled RNA found in the 16 to 27S region, 73% migrated as a homogeneous band in the 27S position of the gel, probably representing single-stranded viral RNA extracted from mature phage.

Effect of virus infection on size distribution of labeled RNA. The pulse-chase labeling technique was used to characterize the size distribution of the heterogeneous 5 to 15S RNA of infected cells. Cells were pulse-labeled for 15 sec with ^3H -uridine at various times after infection and then chased

FIG. 4. *Distribution of ^3H -uridine-labeled RNA associated with polysomes from virus-infected cultures and control cultures after a chase with unlabeled uridine. Both cultures were pulse-labeled for 15 sec with ^3H -uridine at 45 min after infection and then chased by adding a 100-fold excess of unlabeled uridine 5 min before harvesting. Polysome extracts were prepared; labeled lysate (2.8×10^5 counts/min) was layered on a 10 to 40% sucrose gradient and centrifuged at 40,000 rev/min for 70 min at 2°C in a Beckman SW41 rotor. The ratio of the counts per minute per optical density unit (at 260 nm) of the lysate from the control culture to the infected culture was 0.98 after a 5-min chase. The numbers 1, 2, and 3 indicate the position of the monomers, dimers, and trimer, respectively. Roman numerals I, II, and III represent the tetramers and larger polysomes, monomers to trimers, and ribosomal subunit-soluble regions of the polysomes pooled for subsequent experiments. Symbols: uninfected (●) and R17 phage-infected (■) cells.*

FIG. 5. Polyacrylamide gel electrophoresis profiles of ^3H -uridine-labeled RNA associated with polysomes,

for 10 min with a 100-fold excess of unlabeled uridine. The labeled RNA was phenol-extracted and analyzed by polyacrylamide gel electrophoresis. Labeled RNA migrating between the 4 and 16S RNA bands was pooled in two fractions containing small- or large-sized RNA by dividing the fractions in half between the 4 and 16S RNA bands. The ratio of small to large RNA remained unchanged during the initial 15 min of virus infection (Table 4). Between 15 and 45 min after infection, the size distribution of the RNA was progressively altered, resulting in a larger amount of the smaller species of labeled RNA.

DISCUSSION

Infection of *E. coli* with the bacteriophage R17 results in both early and late inhibition of protein synthesis. The early period of inhibition results from an alteration in permeability of the cell caused by the attachment of the phage to the F pili (19, 20). The late inhibition requires the production of both viral polymerase and viral RNA (14) and involves a shift from synthesis of cellular proteins to synthesis of viral proteins (18). As cellular RNA transcription does not appear to be impaired, with the exception of rRNA (2, 8; Iglewski, *submitted for publication*), the site of inhibition of protein synthesis probably is at the translational level.

Infection with the R17 phage results in an alteration of the absorbance profile of polyribosomes characterized by a decrease in the number of large polysomes and an increase in the number of smaller polysomes (5, 14). Although the altered polysome distribution does not account for the shift from host to viral protein synthesis, it appears to be associated with the overall decrease in protein synthesis (5). An understanding of how the polysome shift is induced may provide insight into the inhibition of protein synthesis. Possible mechanisms which might account for a decrease in larger size polysomes are: (i) polysome degradation, (ii) inhibition of polysome formation, or (iii) disaggregation of polysomes. The second

after a chase with unlabeled uridine. Uninfected cultures and R17 phage-infected cultures were pulse-labeled with ^3H -uridine for 15 sec and then chased with a 100-fold excess of unlabeled uridine 5 min before harvesting. Polysomes were extracted and sedimented in a sucrose gradient, and the labeled RNA from the polysomes was analyzed by polyacrylamide gel electrophoresis as described in Fig. 3. An RNA preparation containing 4, 16, and 23S *E. coli* RNA and purified viral RF and RI was used as migration markers in the gels. The distribution of labeled RNA from the region of the polysome profile of uninfected cells and R17-infected cells containing (a) four or more ribosomes per mRNA, (b) one to three ribosomes per mRNA, and (c) ribosomal subunit-soluble region is shown.

TABLE 3. *Distribution of labeled RNA in polysomes from cells pulse-labeled with ^3H -uridine for 15 sec and then chased for 5 min with unlabeled uridine at 45 min after infection^a*

RNA fraction	Labeled RNA associated with polysomes (%)					
	I ^b (>3 ribosomes/mRNA)		II ^b (1-3 ribosomes/mRNA)		III ^b (<70S)	
	Uninfected cells	Virus-infected cells	Uninfected cells	Virus-infected cells	Uninfected cells	Virus-infected cells
4S RNA	4.4	0.9	5.8	2.2	54.7	22.0
Small mRNA	2.0	1.5	4.4	5.3	3.8	12.9
Large mRNA	11.5	4.1	6.3	23.9	2.6	14.7
16S-27S RNA	77.4	37.6	79.9	267.3	34.7	23.4
Very large RNA	4.7	2.2	3.6	6.2	4.2	2.1
Viral RNA		12.7		4.6		1.9
Totals	100.0	59.0	100.0	309.5	100.0	77.0

^a Correction factors (see Table 2, footnote *a*) for regions I, II, and III are 10.6/17.9, 33.8/10.9, and 53.6/69.2, respectively (derived from values given in Table 1, under "15-sec pulse-label preceding 5-min chase").

^b Roman numerals represent the polysome regions shown in Fig. 4.

TABLE 4. *Size distribution of ^3H -uridine-labeled RNA after 10-min chase with unlabeled uridine*

Time after infection (min)	Small (5-9S) RNA (%)	Large (10-15S) RNA (%)
0 (Uninfected)	38.5	61.5
5	39.2	60.8
15	39.4	60.6
35	42.3	57.7
45	50.6	49.4

possibility was suggested by Phillips et al. (14), who reported that the inhibition of polysome formation in R17-infected cells appears to result from the blockage of mRNA translation after the formation of the initiation complex. This inhibition of mRNA translation results in an accumulation of polysomal monomers. The third possibility, the disaggregation of polysomes, has been reported to occur in cells infected with poliovirus (11). This disaggregation appears to result from an inhibition of protein synthesis by double-stranded poliovirus RNA (1, 9). A disaggregation of polysomes, similar to that observed in poliovirus-infected cells, may occur after infection with the R17 phage. This similarity is suggested by the association of viral polymerase and viral RNA synthesis with the disappearance of large polysomes (14). Synthesis of double-stranded R17 RNA might be required for the inhibition of protein synthesis.

In this report, the distribution of labeled RNA associated with polysomes was studied to provide further information on the mechanism involved

in altering the polysome distribution of R17-infected cells. Pulse-labeling of cells from 15 sec results in a greater amount of labeled RNA association with larger polysomes of infected cells, as compared to control cells. Analysis of the polysome-associated RNA indicates that the increased radioactivity in the region of the large polysomes results from the association of labeled double-stranded viral RNA with the polysomes. In addition, 15-sec pulse-labeled species of cellular RNA appear capable of entering into the formation of polysomes in infected cultures and control cultures. The data suggest that rapidly labeled, newly synthesized species of cellular RNA are at least initially capable of entering into the formation of polysomes in infected cells. This phenomenon might be similar to the delay observed in the disaggregation of polysomes in reticulocyte lysates in the presence of poliovirus double-stranded RNA (9). In the reticulocyte system, several rounds of polypeptide chain synthesis occur before polysome disaggregation. That the protein-synthesizing machinery of an R17-infected cell appears capable of accepting newly synthesized mRNA initially to form polysomes suggests that the mRNA is subsequently altered to render it inactive, a concept that might involve binding of factors to the mRNA. This line of reasoning is not without precedent; at least viral mRNA translation may be modified or inhibited by the binding of viral coat protein or viral polymerase to the message (10, 16, 17).

Pulse-labeling of R17-infected cells for 90 sec results in a labeled RNA distribution similar in appearance to the absorbance profile of the poly-

somes. A greater amount of radioactivity is associated with smaller polysomes than with large polysomes. This type of labeling pattern possibly reflects the increased labeling of ribosomes that contribute mainly to the absorbance profile of polysomes (11), or to relocation of labeled RNA from the larger to the smaller polysome region of the sucrose gradient, or to both. The latter possibility appears to be true, as, under pulse-chase conditions, there is a decrease in labeled RNA associated with large polysomes and an increase in the amount associated with smaller polysomes. Analysis of the labeled RNA indicates that the amount of cellular RNA found in the larger polysome region of the sucrose gradient decreases to a greater extent in infected cells than in control cells. The increase in labeled 5 to 15S RNA found in the monomer to trimer region and in the ribosomal subunit-soluble region of the polysome profile of infected cells suggests that the RNA from large polysomes has been relocated in these two regions. The shift in location of the labeled RNA, together with the altered absorbance profiles of polysomes, possibly results from the degradation or disaggregation of larger polysomes. This possibility is suggested by the increase in number of larger mRNA molecules found in the monomer to trimer region of the polysomes. Had polysome degradation occurred, one would expect the labeled 5 to 15S RNA found in this region to be degraded (13). The increased amount of 5 to 15S RNA found in the ribosomal subunit-soluble region of polysomes from infected cells suggests that the RNA may be prevented from entering into the formation of polysomes.

An examination of the size distribution of the heterogeneous 5 to 15S RNA, extracted directly from cells rather than from a specific polysome region, suggests that RNA degradation occurs during virus infection. The size distribution of the heterogeneous RNA extracted from cells during the first 15 min after infection is not altered. Thereafter, a progressively increasing amount of smaller RNA is observed. The size distribution of the 5 to 15S RNA extracted from cells at 45 min after infection (Table 4) is similar to the distribution of 5 to 15S RNA in the ribosomal subunit-soluble region of the polysome profile (Table 3). This similarity might be expected, as the major proportion of labeled RNA in the polysome extract is found in this region (Table 1). The alteration in size distribution of the 5 to 15S RNA appears to correlate with the time reported for the alteration in polysome distributions (5). The apparent degradation of RNA possibly represents the ultimate fate of the RNA

that cannot enter into polysome formation. This suggestion is supported by the apparent accumulation of 5 to 15S RNA in the ribosomal subunit-soluble region of the polysomes and by the data of Phillips et al. (14), indicating that altered polysomes result from inhibition of mRNA translation, not from degraded polysomes.

There are a number of possible mechanisms for the inhibition of protein synthesis in infected cells. The accumulation of data will enlarge our view of potential control mechanisms. In the particular system being studied, further analysis is required to establish the mechanism or integration of mechanisms involved in the inhibition of host protein synthesis.

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LITERATURE CITED

1. Ehrenfeld, E., and J. Hunt. 1971. Double-stranded poliovirus RNA inhibits initiation of protein synthesis by reticulocyte lysates. *Proc. Nat. Acad. Sci. U.S.A.* 68:1075-1078.
2. Ellis, D. B., and W. Paranchych. 1963. Synthesis of ribonucleic and protein in bacteria infected with an RNA bacteriophage. *J. Cell. Comp. Physiol.* 62:207-213.
3. Franklin, R. M. 1966. Purification and properties of the replicative intermediate of the RNA bacteriophage R17. *Proc. Nat. Acad. Sci. U.S.A.* 55:1504-1511.
4. Granboulan, N., and R. M. Franklin. 1966. High resolution autoradiography of *Escherichia coli* cells infected with bacteriophage R17. *J. Bacteriol.* 91:849-857.
5. Hotham-Iglewski, B., and R. M. Franklin. 1967. Replication of bacteriophage ribonucleic acid: alterations of polyribosome patterns and association of double-stranded RNA with polyribosomes in *Escherichia coli* infected with bacteriophage R17. *Proc. Nat. Acad. Sci. U.S.A.* 58:743-749.
6. Hotham-Iglewski, B., L. A. Phillips, and R. M. Franklin. 1968. Viral RNA transcription-translation complex in *Escherichia coli* infected with bacteriophage R17. *Nature (London)* 219:700-703.
7. Hudson, J. B., and W. Paranchych. 1967. Effect of bacteriophage R17 infection on host directed synthesis of ribosomal ribonucleates. *J. Virol.* 2:529-537.
8. Hudson, J. B., and W. Paranchych. 1968. Synthesis of soluble ribonucleates in *Escherichia coli* infected with bacteriophage R17. *J. Virol.* 2:124-128.
9. Hunt, T., and E. Ehrenfeld. 1971. Cytoplasm from poliovirus-infected HeLa cells inhibits cell-free hemoglobin synthesis. *Nature N. Biol.* 230:91-94.
10. Kolakofsky, D., and C. Weissman. 1971. Possible mechanism for transition of viral RNA from polysome to replication complex. *Nature N. Biol.* 231:42-46.
11. Penman, S., K. Scherrer, Y. Becker, and J. E. Darnell. 1963. Polyribosomes in normal and poliovirus-infected HeLa cells and their relationship to messenger RNA. *Proc. Nat. Acad. Sci. U.S.A.* 49:654-661.
12. Phillips, L. A., B. Hotham-Iglewski, and R. M. Franklin. 1969. I. Effects of monovalent cations on the distribution of polysomes, ribosomes and ribosomal subunits. *J. Mol. Biol.* 40:270-288.
13. Phillips, L. A., B. Hotham-Iglewski, and R. M. Franklin. 1969. Polyribosomes of *Escherichia coli*. II. Experiments to

- determine the *in vivo* distribution of polysomes, ribosomes and ribosomal subunits. *J. Mol. Biol.* 45:23-38.
14. Phillips, L. A., J. L. Truden, W. J. Iglewski, B. Hotham-Iglewski, and R. M. Franklin. 1969. Replication of bacteriophage ribonucleic acid: alternating in polyribosome patterns in *Escherichia coli* infected with amber mutants of bacteriophage R17. *Virology* 39:781-790.
 15. Pons, M. W., and G. K. Hirst. 1968. Polyacrylamide gel electrophoresis of influenza virus RNA. *Virology* 34:386-388.
 16. Robertson, H., R. E. Webster, and N. Zinder. 1968. Bacteriophage coat protein as repressor. *Nature (London)* 218:533-536.
 17. Sugiyama, T., and D. Nakada. 1968. Translational control of bacteriophage MS2 RNA cistrons by MS2 coat protein: polyacrylamide gel electrophoretic analysis of proteins synthesized *in vitro*. *J. Mol. Biol.* 31:431-440.
 18. Sugiyama, T., and H. O. Stone. 1968. Viral and bacterial protein synthesis in MS 2 infected *Escherichia coli* cells. *J. Mol. Biol.* 36:91-105.
 19. Watanabe, H., and M. Watanabe. 1970. Expression of the lac operon in *Escherichia coli* infected with RNA phage R23. *Can. J. Microbiol.* 16:713-717.
 20. Yamazaki, H. 1969. The mechanism of early inhibition by an RNA phage of protein and RNA synthesis in infected cells. *Virology* 37:429-436.