Polysomal Localization of R17 Bacteriophage-Specific Protein Synthesis

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Synthesis of viral ribonucleic acid (RNA) polymerase, maturation protein, and coat protein in *Escherichia coli* infected with bacteriophage R17 occurs mainly on polysomes containing four or more ribosomes. The 30S ribosomal subunits through trimer-size polysomes, which are associated with all of the R17-specific proteins and are predominant in the infected cell, synthesize only coat protein. These structures may accumulate as products derived from larger polysomes as a result of failure in the release of nascent polypeptides after termination of chain growth. Appreciable amounts of viral coat protein remain attached to ribosomes and polysomes during R17 bacteriophage replication, supporting the hypothesis of the repressor role of this protein. The time course of synthesis of virus-specific proteins obtained from the polysomes of infected cells demonstrated regulated R17 messenger RNA translation consistent with the idea that coat protein is preferentially synthesized whereas the synthesis of noncoat proteins is suppressed.

R17 bacteriophage ribonucleic acid (RNA) can act as its own polycistronic messenger in infected *Escherichia coli* cells, resulting in the production of capsid, RNA synthetase, and maturation proteins (11). Both parental and progeny viral RNA can be found in the polysomal fraction (10). Consequently, virus-specific protein synthesis should be found associated with some of the structures containing the protein-synthesizing machinery of the host cell.

An alteration in the size distribution of host cell polysomes occurs after infection of *E. coli* with R17 bacteriophage (12). As the infection cycle proceeds there occurs a progressive decrease in the amount of polysomes of hexamer size or larger, a predominance of trimers through pentamers, and an increase in 70S monomers. Concomitantly, there is a decrease in the labeling of polypeptides with radioactive amino acids in the larger polysomes, and most of the labeled protein is now found in the smaller ones, rather than in polysomes containing four to eight ribosomes, as occurs in uninfected cells. The trimers through pentamers may represent that polysome fraction(s) in which R17 bacteriophage-specific protein synthesis is localized. Double-stranded RNA or replicative intermediate is prominent, however, in the polysomes of pentamer and

hexamer size or larger, rather than in the smaller ones (9, 10, 12, 13). Thus, the regions in which one finds viral replicating structures are not the regions in which viral protein synthesis is apparently predominant.

The findings cited above point out the need for further study of the synthesis and control of virus-specific proteins in R17-infected *E. coli* cells. Accordingly, experiments were performed to answer the following questions. Are the polysomes in the trimer to pentamer region those responsible for the synthesis of R17 bacteriophage-specific protein(s)? If so, which of the major viral proteins are made here? What is the significance to bacteriophage replication of any pattern of polysome localization with respect to virus-specific protein synthesis?

MATERIALS AND METHODS

Bacteria, media and solutions, and bacteriophage. Standard procedures for the maintenance of *E. coli* 3000 and Q-13 cells and R17 bacteriophage have been previously described (22).

Stock cultures of strain 3000 were prepared monthly in MS broth from freshly isolated colonies. Each isolate was tested for the presence of contaminating bacteriophage and for the F⁺ characteristic before use in an experiment.

Single colonies of *E. coli* Q-13 were tested for the lack of ribonuclease I and phosphorylase by the method of Gesteland (8). To avoid higher levels of nuclease activity, due to the accumulation of wild-

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type revertants upon serial propagation of this mutant, only the first passage after single colony isolation was used in the preparation of cell-free extracts. First passages of Q-13 stocks, containing a final concentration of 25% glycerol, were quick-frozen in dry ice-acetone and stored at -70°C .

An enriched medium was used for the growth of Q-13 cells in the preparation of cell-free extracts. It consisted of 32% tryptone, 20% yeast extract, 5% NaCl, and 0.005 N NaOH (17). Under vigorous aeration, a 1:50 dilution of freshly thawed Q-13 stock cells was incubated in this medium at 25°C for 6 hr [optical density at 610 nm (OD_{610}) = 0.4, end of log phase], yielding up to 5 g wet weight of cells from 1 liter of culture instead of the usual yield of 0.5 g.

Fresh preparations of stock R17 bacteriophage, from MS broth-grown 3000 cells, were prepared every 3 to 4 months depending upon the extent of loss of infectious titer as determined by plaque assay. Average titers of fresh preparations ranged from 0.8×10^{12} to 1.2×10^{12} plaque-forming units (PFU) per ml. These preparations were always reassayed shortly before use.

General procedures. All procedures concerning the preparation of tris(hydroxymethyl)aminomethane (Tris)-magnesium-sodium (TMNa^+) cell lysates as a source of polysomes, sucrose gradient centrifugation of lysates, and subsequent fractionation of gradients were done by the methods of Phillips et al. (23), with the exception that in the present study 15 to 28% isokinetic gradients (19), instead of 10 to 40% exponential gradients, were used. For the determination of radioactivity profiles, 50- μl samples of each gradient fraction were precipitated with cold trichloroacetic acid and counted as previously described (22).

Extraction of proteins for analysis on polyacrylamide gels, the electrophoresis, cutting of gels, processing of gel slices for radioactivity counting, and the counting were as previously described (24).

S-100 extracts were prepared from *E. coli* Q-13 cells by the method of Iwasaki et al. (14). Small samples of S-100 were quick-frozen in dry ice-acetone, stored at -70°C , and thawed just before use.

In vivo experimental design. A fresh overnight MS broth culture of *E. coli* 3000 cells was diluted 1:25 in 100 ml of TCGI (synthetic) medium (12, 22, 23) for each ensuing polysome preparation. The cells were grown at 37°C to a density of 2×10^8 cells/ml (OD_{610} = 0.23, early log phase) and then were centrifuged at room temperature for 10 min at $5,000 \times g$. The drained cell pellet was resuspended in a sufficient volume of stock R17 bacteriophage (usually 0.2 to 0.4 ml) to provide an input multiplicity of infection equal to 10. Adsorption of bacteriophage to cells was allowed to proceed for 5 min at room temperature. The infected cells were then quickly transferred to 50 ml of prewarmed TCGI (containing 0.01 the usual amount of Casamino Acids) to give a density of 4×10^8 cells/ml, and incubation at 37°C was begun. This step was considered to be the start of the infection period, or "time zero."

At 5 min postinfection rifampin was added to a level of 45 $\mu\text{g}/\text{ml}$. To separate cultures a mixture of ^{14}C -L-amino acids was added to a final concentration of 2 $\mu\text{Ci}/\text{ml}$ at 10, 15, 20, 30, and 40 min, respectively.

Incorporation was stopped 3 min later by quickly pouring the cells over an equal volume of frozen, crushed TCGI containing 0.3 mM chloramphenicol and 10 mM sodium azide.

In displacement experiments, cells infected for 20 min were labeled as just described, but immediately after the 3-min pulse time had elapsed, either puromycin (final concentration, 2.5 mg/ml) or a 1,000-fold excess of ^{14}C -L-amino acids was added, and incubation was allowed to continue for another 5 min, so that these cells were harvested at 28 min postinfection. The high concentration of puromycin used in these experiments was necessitated by the presence of rifampin. In its absence, 250 μg of puromycin per ml effectively inhibited protein synthesis. Rifampin did not interfere with the action of either chloramphenicol or tetracycline, the latter being used at a level of 100 $\mu\text{g}/\text{ml}$.

In all cases, just before the cells were poured into frozen TCGI, 1 to 2 ml of cell suspension was transferred to ice-chilled containers to provide whole-cell controls for monitoring the infection process with respect to the production of bacteriophage-specific proteins.

Lysates for polysomes were prepared from the harvested cells and fractionated as mentioned above. A lysate from uninfected, unlabeled cells served as a quality control for each polysome preparation. The OD_{260} profile of this lysate after sucrose gradient fractionation showed whether or not one could expect a representative yield of polysomes. In the case of labeled lysates, the portion of each gradient fraction remaining after removal of a 50- μl sample for radioactivity assay was frozen and stored at -20°C .

On the basis of the radioactivity profile of each gradient, six pools were made from the fractions according to approximate particle size likely to be found in a given region of the gradient. Pool I contained polysomes of approximately octamer and larger size; pool II, hexamers and septamers; pool III, tetramers and pentamers; pool IV, dimers and trimers; pool V, 30 through 80S particles; and pool VI, the soluble fraction of cell lysates. The proteins were extracted from these pools and analyzed by polyacrylamide gel electrophoresis.

In vitro experimental design. Up to the point including the fractionation of cell lysates by sucrose gradient centrifugation, the protocol used in the in vitro experiment was identical to that of the in vivo study with a 20-min infection period. In the in vitro work all sucrose solutions were supplemented with 0.5 mM dithiothreitol in an effort to stabilize the polypeptides associated with ribosomes and polysomes during centrifugation.

Pools similar to those in the in vivo work were made from throughout the polysome and subunit-containing regions of the sucrose gradient, but a pool of the soluble fraction of cell lysates was omitted. The amount of ribosomal and polysomal material in these pools was not determined.

Sufficient quantities of the following ingredients were added to each 2.5-ml pool (held at 2°C) so that the final concentration of reactants in a total volume of 5.5 ml incubation mixture was: 75 μM folinic acid; 750 μg of transfer RNA (tRNA) per ml; 60 mM Tris-

hydrochloride buffer (pH 7.8); 45 mM KCl; 14 mM magnesium acetate; 1.1 mM adenosine triphosphate (ATP); 0.25 mM guanosine triphosphate (GTP); 9 mM phosphoenol pyruvic acid (PEP); 20 μ g of phosphoenol pyruvic acid kinase (PEP-kinase) per ml; 18 mM 2-mercaptoethanol (2-ME); 0.1 mM of each of 20 ^{12}C -L-amino acids, and 1.1 mg of S-100 protein per ml.

The reaction vessels were quickly transferred to a water bath at 37 C and incubated for 1 hr. The reaction was terminated by pouring the incubation mixture onto an equal volume of frozen, crushed TCGI containing 0.3 mM chloramphenicol and 10 mM sodium azide.

One-half of each incubation mixture was layered onto 15% (w/v) sucrose containing a 60% (w/w) sucrose cushion and centrifuged for 6 hr at $100,000 \times g$. The top 6 ml of each sucrose column was collected for free polypeptides, and the bottom 1.5 ml for polypeptides bound to ribosomes and polysomes. All such samples were frozen and stored at -20 C until the proteins therein were extracted and analyzed by polyacrylamide gel electrophoresis.

Sources of materials. Materials and their suppliers were as follows: phenol, Merck Co., Rahway, N.J.; 20 individual ^{12}C -L-amino acids, Mann Research Laboratories, New York, N.Y.; a mixture of ^{14}C -L-amino acids (100 $\mu\text{Ci}/\text{ml}$, 52 to 54 mCi/milliatom carbon) New England Nuclear Corp., Boston, Mass.; ATP (neutralized sodium salt) and GTP (sodium salt), P-L Biochemicals, Inc., Milwaukee, Wis.; PEP-kinase (from rabbit skeletal muscle), Sigma, St. Louis, Mo.; folic acid (calcium leucovorin), Lederle Laboratories, Pearl River, N.Y.; and *E. coli* B tRNA, Schwarz BioResearch Co., Orangeburg, N.Y.

Sources for other materials have been previously indicated (23, 24).

Sodium dodecyl sulfate was recrystallized from ethyl alcohol, but phenol was not redistilled. Acid-washed alumina was prepared from bacteriological grade A 301 alumina (Alcoa Chemicals, Bauxite, Ark.). The monocylohexylammonium form of PEP was prepared and subsequently converted to the potassium salt by the methods of Clark and Kirby (4) and was generously provided by H. S. Penefsky, of this institute.

Rifampin was the kind gift of I. D. Steinman, CIBA Pharmaceutical Co., Summit, N.J.

RESULTS

Bacteriophage-specific proteins from polysomes of cells infected for various periods of time. An examination was made of the bacteriophage-specific proteins associated with various sizes of polysomes taken from cells infected for 10, 15, 20, 30, and 40 min, respectively. Figure 1 shows the ^{14}C -amino acid profile of the sucrose gradients used for fractionation of cell lysates from the 10-, 20-, and 40-min infection periods. The corresponding polyacrylamide gel analyses for the proteins obtained from the various sizes of polysomes are shown in Fig. 2 through 4. Due to the drastic inhibitory effect of rifampin on cell

FIG. 1. Sucrose density gradient centrifugation of lysates from *Escherichia coli* cells infected with R17 bacteriophage for 10, 20, and 40 min. Sedimentation is from right to left. Symbols: (○) 10 min of infection; (△) 20 min of infection; (□) 40 min of infection.

polysome formation, radioactivity, rather than OD_{260} , profiles are shown. Because of some inhibitory effect of rifampin on the growth of R17 bacteriophage, particularly later in the infection cycle, experiments were performed to determine whether the drug could be omitted from the experimental design or added later than 5 min postinfection. Under both of these conditions, greater specific radioactivity labeling of the various bacteriophage-specific proteins should be possible. Unfortunately, the omission of rifampin or its addition after 5 min postinfection permitted sufficient labeling of cellular material so that the resultant high base line of radioactivity precluded clear definition of viral protein peaks in ensuing polyacrylamide gel profiles. Moreover, for clean backgrounds in these gels the antibiotic must be present for at least 10 min before labeling.

As the length of infection time increases, there is a progressive increase in the labeling of the lighter polysome complexes (Fig. 1), reflecting the initial observations of Hotham-Iglewski and Franklin in regard to a polysome shift in infected cells (12). By 20 min of infection there is an increase in labeling of coat protein (Fig. 3). After 20 min of infection, relative labeling of viral RNA polymerase and maturation protein diminishes, and by 30 or 40 min coat protein is predominant (Fig. 4). All sizes of polysomes

FIG. 2. Electropherograms of proteins associated with various polysome fractions sedimenting in the sucrose gradient charged with lysate from *Escherichia coli* cells infected with R17 bacteriophage for 10 min. Extraction and electrophoresis of proteins were as previously described (33) except that our running buffer contained 1 mM dithiothreitol in addition to phosphate buffer and sodium dodecyl sulphate. Vertical arrows indicate positions of the three major bacteriophage-specific proteins (γ , viral RNA polymerase; α , maturation protein; β , coat protein) in the internal migration marker, which consisted of ^3H -leucine-labeled proteins extracted from rifampin-treated, infected, whole cells. These proteins were identified by comparison of the pattern of labeled proteins from cells infected with wild-type virus with the pattern from cells infected with appropriate amber mutants of bacteriophage R17 (cf. 33). Amber mutants were kindly provided by K. Weber, Harvard University. Migration is from left to right. (a) Octamers and larger; (b) hexamers and septamers; (c) tetramers and pentamers; (d) dimers and trimers; (e) 30 through 70S particles; (f) soluble fraction of lysate.

reflected the same bacteriophage-specific protein pattern for a given time period of infection, and all sizes of polysomes yielded all three bacteriophage-specific proteins. The gel patterns of the proteins isolated from infected, whole cells (not shown) were identical to those for the proteins isolated from the polysomes of infected cells.

In vivo displacement of bacteriophage-specific polypeptides from polysomes in infected cells. The results presented in the previous section suggested to us that in *E. coli* cells infected with R17 bacteriophage all sizes of polysomes may be synthesizing all three major bacteriophage-specific

proteins, assuming that all of these proteins are nascent. This assumption may not be valid, because some of these proteins could be completed polypeptides serving regulatory or enzy-

FIG. 3. Electropherograms of proteins obtained with a 20-min infection period in the experiment depicted in Fig. 1. For details, see legend to Fig. 2.

FIG. 4. Electropherograms of proteins obtained with a 40-min infection period in the experiment depicted in Fig. 1. For details, see legend to Fig. 2.

matic functions, such as coat protein and viral RNA polymerase, respectively. Indeed, it has been suggested that coat protein may act as a repressor in regulating the synthesis of the polymerase and maturation protein (30).

Therefore, we determined whether or not the bacteriophage-specific polypeptide chains could be displaced from the polysomes of infected cells. The means by which we attempted to displace the proteins *in vivo* consisted of chase with either cold amino acids or puromycin. Chloramphenicol was included for comparative purposes since it does not displace nascent polypeptides from polysomes. Figures 5 through 11 show the results of the *in vivo* displacement experiments. A comparison of the relative proportions of the three viral proteins seen in the chase gel patterns with those seen in the time-labeling control patterns shows whether inhibition of protein synthesis or displacement of labeled polypeptides, or both, has occurred. A 3-min time labeling control is that period of labeling in infected, untreated cells before the start of the chase treatment in infected, treated cells; and the 8-min control was comprised of this 3-min pulse plus an additional 5 min of labeling so that label was present for the same length of time in both treated and untreated cells. In the 3-min labeling control, the ratio between viral RNA polymerase

FIG. 5. Sucrose density gradient centrifugation of lysates from *Escherichia coli* cells infected with R17 bacteriophage for 20 min with subsequent treatment to displace nascent polypeptides. Symbols: (○) 8-min labeling control; (●) 3-min labeling control; (▼) puromycin treatment; (△) chloramphenicol treatment; (□) cold amino acid chase.

FIG. 6. Electropherograms of proteins from octamers and larger polysomes sedimenting in the sucrose gradients charged with lysates from variously treated cells—indicated in Fig. 5. (a) 8-min labeling control; (b) 3-min labeling control; (c) puromycin treatment; (d) chloramphenicol treatment; (e) cold amino acid chase.

and maturation protein is consistently about 2:1 for all sizes of polysomes, whereas coat protein increases from 2 times the amount of maturation protein in the largest polysomes up to about 6 times in the dimers and trimers. These ratios for the 8-min control are more nearly 1:1 for the polymerase and maturation protein, with coat protein increasing from 4 up to 10 times the amount of maturation protein as one proceeds from the largest to the smallest polysomes. It was possible that displacement of all labeled peptides would not occur during the chase period, but rather there would occur inhibition only of further synthesis of bacteriophage-specific proteins during this period. In that case the resultant gel pattern should more nearly resemble the pattern for the 3-min labeling control, rather than the 8-min control, with respect to the relative proportions of the three bacteriophage-specific proteins. Although puromycin failed to displace the peptides labeled before its addition, the antibiotic did stop further synthesis of viral polypeptides during the subsequent 5-min treatment period, presumably by displacement of these peptides according to the accepted mode of puromycin action (1). This is further indicated

FIG. 7. Electropherograms of proteins from hexamers and septamers obtained in the experiment depicted in Fig. 5. For details, see legend to Fig. 6.

FIG. 9. Electropherograms of proteins from dimers and trimers obtained in the experiment depicted in Fig. 5. For details, see legend to Fig. 6.

FIG. 8. Electropherograms of proteins from tetramers and pentamers obtained in the experiment depicted in Fig. 5. For details, see legend to Fig. 6.

FIG. 10. Electropherograms of proteins from 30 through 70S-size particles obtained in the experiment depicted in Fig. 5. For details, see legend to Fig. 6.

FIG. 11. Electropherograms of proteins from the soluble fraction of lysate remaining at the top of the sucrose gradients depicted in Fig. 5. For details, see legend to Fig. 6.

by the appearance of viral-specific polypeptides in the soluble fraction of the cell lysate (Fig. 11c). As expected, according to the known mechanism of chloramphenicol action (3), this drug also inhibited further synthesis of viral peptides but caused little, if any, displacement of them from the polysomes. On the contrary, more viral peptides appear to be associated with the polysomes of infected, chloramphenicol-treated cells than with the polysomes of otherwise-treated, infected cells (Fig. 6d through 10d). This can be explained by the ability of this drug to prevent ribosomal run-off from polysomes, thus "freezing" peptides at their site of formation (18).

The cold amino acid chase almost entirely displaced viral RNA polymerase and maturation protein from the polysomes, especially in the size class greater than tetramers, but it failed to dislodge very much coat protein. It can be seen that the soluble fraction of this cell lysate contains ample amounts of both of the former proteins, but comparatively little coat protein (Fig. 11e).

In vitro displacement of bacteriophage-specific polypeptides from polysomes obtained from infected cells. A second procedure which we used in studying the nature of the bacteriophage-specific proteins detected in vivo on the poly-

somes of infected cells consisted of determining whether or not these proteins, labeled in vivo during the ^{14}C -amino acid pulse, could subsequently be completed, and hence detached from the polysomes, when these latter complexes were put into a cell-free protein-synthesizing system. Under these conditions it was found that polysomes of approximately tetramer size, or larger, appeared to be the primary size class of polysomes responsible for the synthesis of all three major R17 bacteriophage-specific proteins. This was indicated by the almost complete displacement of ^{14}C -labeled viral RNA polymerase and maturation protein from polysomes of these sizes along with partial displacement of coat protein (Fig. 12 through 14 and Table 1). No such displacement of polymerase and maturation protein occurred in the case of dimers and trimers or the 30 to 80S pools, and hence these structures do not seem to be engaged in synthesizing these proteins. Large amounts of coat protein appeared in the supernatant fluid of the respective in vitro reaction mixtures in which 30S through trimer-size polysomes were incubated (Fig. 13).

FIG. 12. Electropherograms of proteins from cell-free protein-synthesizing mixtures containing various polysome fractions previously sedimented in a sucrose gradient charged with lysate from *Escherichia coli* cells infected with R17 bacteriophage for 20 min. Details of the various determinations were the same as in Fig. 2, except that in the present experiment the soluble fraction of the lysate was not tested.

Taken collectively, the results of both the *in vivo* (cold chase) and *in vitro* displacement experiments indicate that, whereas almost the entire population of viral RNA polymerase and maturation protein molecules detected on the larger

FIG. 13. Electropherograms of proteins from supernatant fluid of polysomes present in mixtures indicated in Fig. 12.

TABLE 1. Per cent distribution of R17-specific proteins between pelleted ribosomes and polysomes and their supernatant fluids after incubation in a cell-free protein-synthesizing system^a

Particle size	Viral RNA polymerase (%)		Maturation protein (%)		Coat protein (%)	
	Pellet	Super-natant fluid	Pellet	Super-natant fluid	Pellet	Super-natant fluid
Octamers and larger.....	8	92	12	88	22	78
Hexamers and septamers...	16	84	19	81	35	65
Tetramers and pentamers...	27	73	35	65	33	66
Dimers and trimers.....	81	19	85	15	50	50
30 through 70S.....	95	5	86	14	72	28

^a Values derived from the sum of counts/minute under the various peaks in Fig. 13 and 14.

FIG. 14. Electropherograms of proteins from ribosomes and polysomes present in mixtures indicated in Fig. 12.

polysomes of infected cells may be nascent polypeptides, coat protein may consist of completed chains in addition to nascent ones. Furthermore, polysomes of tetramer size or greater are the polysomes actively synthesizing R17 bacteriophage-specific proteins.

DISCUSSION

Synthesis of the three virus-specific proteins found in R17-infected *E. coli* cells occurs mainly on polysomes of tetramer size, or larger. The significance of this finding lies in its relationship to the distribution of replicative intermediate (RI) in infected cells. According to Weissmann and his collaborators (34), RI probably does not exist in the actual bacteriophage replicating complex (RC_R) but rather is formed during extraction from the single-stranded RNA structures involved in replication. Nevertheless, RI has repeatedly been found during the synthesis of RNA bacteriophage viral RNA (6, 7, 21), and we may therefore regard it, although with reservation, as a legitimate means of monitoring replication of the bacteriophage genome.

Those polysomes which contain most of the RI (13) are also the ones primarily responsible for the synthesis of viral RNA polymerase, maturation protein, and coat protein. This supports the suggestion of Hotham-Iglewski et al.

(13) that transcription of the R17 viral genome may be coupled to its translation. Perhaps ribosomes can attach to the free 5' end of the nascent viral RNA present in the RI complex (5) after only a few nucleotides have been polymerized so that translation of the viral messenger may begin almost immediately after initiation of its transcription (29).

The 30S through trimer-size particles that predominate in R17-infected *E. coli* cells (12) do not constitute major synthetic sites for the viral proteins. Their association with all of the R17-specific proteins could represent the end result of failure in the release of completed viral polypeptides after termination of chain growth has occurred in the larger polysomes (25). Several possible mechanisms might be operative in this process. In one case, the 70S particles released from these polysomes may not dissociate since peptidyl chains stabilize the ribosomal complex (28). On the other hand, the monosomes might dissociate, with the nascent peptides remaining on the 50S subunit (26). Upon reassociation of the derived 50S particles with new 30S partners (32), the nascent peptides would prevent binding of f-met tRNA to the 30S-messenger complex (20) so that imperfect initiation complexes would form. The accumulation of aberrant 70S initiation monosomes in R17-infected cells has been previously suggested (24). Dimers and trimers could form through attachment of aberrant ribosomes to one or two of the remaining initiation sites on the bacteriophage messenger RNA (mRNA) (2, 16). These events would result in the accumulation of 30S through trimer-size particles bearing R17 virus-specific polypeptides. Because rifampin starves cells of mRNA, it also causes the disappearance of polysomes, with concomitant accumulation of subunits (27). Because the drug does not interfere directly with any step in protein synthesis (35), nascent peptides should be discharged before release of the ribosomal particle from the polysome.

In the present work, some of the coat protein polypeptides labeled *in vivo* could be displaced from the 30 through 70S ribosomal particles and from the dimers and trimers when they were incubated in the *in vitro* protein-synthesizing system. None of the prelabeled polymerase or maturation protein could be similarly displaced, so that translation impaired beyond the reinitiation step is perhaps restricted to these cistrons on the bacteriophage mRNA. This would be consistent with the repressor role that coat protein may play in RNA bacteriophage replication wherein synthesis of noncoat proteins is suppressed by coat peptides while its own synthesis

proceeds normally, or even at an enhanced rate (30, 31).

Repressor activity of coat protein is further suggested by the fact that in both our *in vivo* cold chase and *in vitro* displacement experiments an appreciable amount of this viral protein always remained associated with ribosomes and polysomes. The particularly large amount of coat protein found associated with 30S subunits through trimers in the *in vitro* experiment may be due to the presence of 80S mature virions, since they are not clearly resolved by our sucrose density gradient centrifugation procedure. Once the coat protein block has been overcome, however, translation should proceed unhindered to the 3' end of the R17 replicating complex. One should then be able to detect polysome-associated synthesis of all of the virus-specific proteins, as shown by the present results obtained for the tetramer-size, or larger, polysomes of R17-infected *E. coli* cells.

A prevalent hypothesis holds that the various RNA bacteriophage proteins are made in different relative proportions throughout the infectious cycle because of the regulatory effect exerted by coat protein (33). Our time course study clearly illustrates temporal control of R17 bacteriophage-specific protein synthesis. Firstly, at 10 min postinfection only viral RNA polymerase and coat protein were being synthesized, which may reflect the mechanism recently proposed by Kolakofsky and Weissmann (15) to account for the conversion of parental bacteriophage RNA from messenger to replicating structure. At 20 to 25 min postinfection, when the shift from large to small polysomes becomes very apparent (12), approximately equal amounts of viral RNA polymerase and coat protein, but somewhat less maturation protein, were found on all polysomes. Later, when few heavy polysomes exist (12), negligible amounts of noncoat proteins were synthesized, and the surviving heavy polysomes made large amounts of coat protein. The changes observed in the relative predominance of the virus-specific proteins with increasing duration of infection suggest that the viral polysomes contain repressed R17 bacteriophage mRNA.

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