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Host-Virus Interaction in Ribonucleic Acid Bacteriophage-Infected *Escherichia coli*

I. Location of "Late" MS2-Specific Ribonucleic Acid Synthesis

ANNE M. HAYWOOD, JANE HARRIS CRAMER,[1] AND NADJA LANCASTER SHOEMAKER[2]

Department of Microbiology, Yale University Medical School, New Haven, Connecticut 06510 and Department of Microbiology, Northwestern University Medical School, Chicago, Illinois 60611

Received for publication 2 June 1969

When actinomycin-treated, MS2-infected *Escherichia coli* are labeled during a brief period later than 16 min after infection, the newly synthesized MS2 ribonucleic acid (RNA) appears first in the 30,000 × *g* sediment, probably bound to fragments of bacterial membranes, since the radioactivity can be released from the sediment with deoxycholate or urea. With longer labeling times, radioactivity also appears in the 30,000 × *g* supernatant fluid. While on the membrane, the RNA is organized into particles with sedimentation coefficients of 40, 32, and 27*S* in the presence of low Mg^{2+}. In the presence of high Mg^{+}, MS2-specific RNA is found in polyribosomes. These data are interpreted to mean that MS2-specific RNA is synthesized and organized into larger structures on membrane. More than 8 min of labeling is required before radioactivity is found in the 81*S* virion which appears in the supernatant fluid.

Much information exists concerning the nature of the protein and ribonucleic acid (RNA) species synthesized during the replication of RNA bacteriophages, but there is little known about the intracellular location of these syntheses. Haywood and Sinsheimer (12) showed that an MS2-specific, histidine-containing protein found in a "41*S*" particle was associated with the sediment obtained after centrifugation at 13,000 × *g* for 10 min, and could be released from the sediment by deoxycholate (DOC). They postulated that this particle contained MS2 RNA polymerase and suggested that MS2 RNA synthesis might take place on "membrane."

This work describes the intracellular location of MS2 RNA which is synthesized later than 16 min after infection. The data are interpreted to mean that MS2-specific RNA is synthesized on membrane and organized into larger structures on membrane.

MATERIALS AND METHODS

Chemicals. Actinomycin-D was a gift from Merck, Sharpe and Dohme Research Laboratories, Rahway, N. J. Uracil-*5*, *6*-^{3}H (27.0 c/mmole) and uracil-*2*-^{14}C (52.8 mc/mmole) were obtained from Schwarz Bio-Research, Inc., Orangeburg, N.Y. Egg white lysozyme and sucrose (density gradient grade) were obtained from Mann Research Laboratories. Deoxyribonuclease I (electrophoretically purified) was obtained from Worthington Biochemical Corp., and from Mann Research Laboratories. Ribonuclease type II-A was obtained from Sigma Chemical Co., St. Louis, Mo. Dithiothreitol was obtained from Calbiochem, Los Angeles, Calif.

Media. *Escherichia coli* was grown in TPG (24) and 0.15% Casamino Acids. $CaCl_2$ was increased to 10^{-3} M and KH_2PO_4 to 0.35 g/liter.

Bacteria and bacteriophage. MS2 was originally obtained from Alvin Clark. *E. coli* D-10, ribonuclease I^{-}, was a gift of Joan Argetsinger Steitz, and was cured of bacteriophage lambda by Raymond Devoret. Phage φX174 and its host *E. coli* C were gifts of Robert L. Sinsheimer.

Preparation of actinomycin-sensitive cells. *E. coli* cells were sensitized to actinomycin as previously described (11), except that the cells were washed only once with 0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 8, and the ethylenediaminetetraacetic acid (EDTA) treatment was shortened to 2 min. Since the characteristics of the sensitization procedure seem to vary according to regional water supply (Haywood, *unpublished observations*), it is advisable to use deionized, triple-distilled water for buffers and reagents.

Harvesting and lysing of cells. To harvest the cells, NaN_3 was added to yield a final concentration of 10 mM, and uracil was added to yield a final concentration of 100 μg/ml. The cells were swirled in a flask placed in dry ice and ethyl alcohol until ice crystals

[1] Present address: Division of Biology, California Institute of Technology, Pasadena, Calif. 91109.

[2] Present address: Biology Department, Northwestern University, Evanston, Ill. 60201.

began to form (5 to 25 sec). All subsequent steps were performed at 0 to 4 C unless otherwise stated. The volumes stated are for 10^{10} to 3×10^{10} cells.

Spheroplasts were made by a variation of the method of Suit (26). The cells were collected by centrifugation and suspended in 0.4 ml of TMKAS (10 mM Tris, *p*H 8, 6 mM $MgCl_2$, 40 mM KCl, 10 mM NaN_3, and 40 mM Na_2SO_4). The cell concentration at this point should be no less than 2×10^{10}/ml, since some cells do not become rounded when the cell concentration is lower during the lysozyme freeze-thaw steps. Lysozyme was added to a final concentration of 1 mg/ml. After 10 min, the cells were frozen and thawed twice, and deoxyribonuclease was added to 10 μg/ml. The cells were left in an ice bath a minimum of 15 min before further fractionation.

In many experiments the lysate was fractionated by centrifugation at 30,000 × *g* for 15 min. The sediment was resuspended in 0.2 ml of TMKAS, and the centrifugation step was repeated. The pooled supernatant fractions from the two steps are referred to as the "supernatant fraction." The sediment from the second step was resuspended in an equal volume (0.6 ml) of TMKAS, and is referred to as the "membrane." Phase-contrast microscopy showed that the membrane consists of nonrefractile "ghosts." When DOC (0.2% final concentration) was added to the sediment, the ghosts disintegrated, and the remaining particulate matter was removed by centrifugation at 30,000 × *g* for 15 min. Centrifugation in the above steps at 10,000 × *g* for 10 min instead of at 30,000 × *g* for 15 min gave identical results.

Trichloroacetic acid precipitation. Trichloroacetic acid precipitation was by a modification of the procedure of Mans and Novelli (21). The samples were put on 3MM Whatman filters which were left for 1 hr in cold 7% trichloroacetic acid and uracil (1 mg/ml). The filters were then washed twice with 5% trichloroacetic acid and uracil (1 mg/ml) and once in absolute alcohol.

RESULTS

When actinomycin-treated, MS2-infected *E. coli* cells are labeled with ^{3}H-uracil at 16 min postinfection or later, the label is in the membrane fraction after very short pulses. Radioactive RNA appears in the supernatant fraction after labeling periods longer than 45 sec at 37 C. To follow the time course of release of viral RNA from the sediment, a culture was labeled for 1.5 generations with ^{14}C-uracil, actinomycin-treated, and divided into infected and uninfected portions; ^{3}H-uracil was added. The uptake of ^{3}H-uracil into the uninfected culture was less than 10% of the uptake into the infected culture. The trichloroacetic acid-precipitable ^{3}H-uracil in the total lysate of samples taken from the infected culture and in the supernatant and membrane fractions was plotted against time after the addition of ^{3}H-uracil (Fig. 1). Corrections for ^{3}H yield were made according to the recovery of ^{14}C. At early times, the uptake of ^{3}H into the membrane fraction is roughly proportional to the time of labeling; whereas the uptake into the supernatant fraction is roughly proportional to the square of the time. This is consistent with a precursor-product relationship (3).

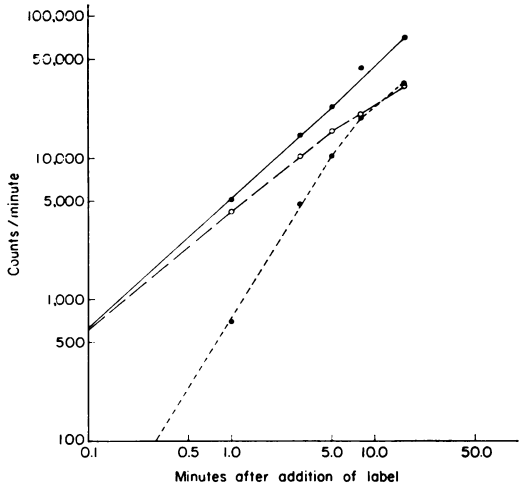

FIG. 1. *Distribution of MS2-specific RNA versus time. E. coli cells were grown in the presence of 1.9 cpm of ^{14}C-uracil per ml (0.32 μg/ml total uracil) for 1.5 doubling times to a concentration of 1.5×10^8 cells/ml. The cells were EDTA-treated and resuspended at a concentration of 6×10^8 cells/ml in growth medium containing actinomycin (0.5 μg/ml). After a 10-min incubation, part of the culture was infected with MS2 at a multiplicity of infection of 5, and the rest was used as an uninfected control. At 18 min after infection, uracil was added to both the infected and uninfected portions to give a concentration of 1 μg/ml. Two minutes later, ^{3}H-uracil was added to 1 μc/ml. At 1, 3, 5, 8, and 16 min after the addition of ^{3}H-uracil for the infected culture and at 3 and 16 min for the uninfected culture, 50-ml samples were removed, harvested, and lysed. Samples of the lysates were then fractionated into 30,000 × g supernatant and membrane fractions. Corrections were made for differences in recovery between samples as determined by the ^{14}C-uracil content. Symbols:* ● *(solid line), trichloroacetic acid-insoluble ^{3}H-uracil in total lysate;* ○ *(long dashed line), trichloroacetic acid-insoluble ^{3}H-uracil in 30,000 × g sediment;* ● *(short dashed line), trichloroacetic acid-insoluble ^{3}H-uracil in 30,000 × g supernatant fraction.*

It is unlikely that the association of newly synthesized MS2-specific RNA with membrane is due to nonspecific trapping. Such trapping would have to be selective for viral RNA, since 40 to 70% of the stable host RNA is in the supernatant fraction. To further check on the possibility of trapping, supernatant fraction from actinomycin-treated, MS2-infected cells labeled for 16 min

was added to a lysate of 10^{10} cells which was subsequently fractionated. Ninety per cent of the radioactivity was found in the supernatant fraction. Centrifugation of the membrane and supernatant fraction on sucrose shows that the supernatant fraction contains no membrane but that a small amount of the supernatant fraction is trapped in the sediment (Haywood, *unpublished data*). A small amount of contamination by the supernatant fraction would result in making the membrane ^3H appear low at early times and high at late times, since the ^3H was corrected for the amount of ^{14}C present, and the ratio of ^3H to ^{14}C in the supernatant fraction is much less at early times and greater at late times than in the membrane fraction.

Release of radioactivity from the membrane. To investigate the type of bonds responsible for association of MS2-specific RNA with the membrane, an experiment similar to that of Fig. 1 was performed using a labeling period of 2.5 min. However, the membrane fraction of both the infected and uninfected cultures was suspended in a variety of solutions and subjected to another cycle of centrifugation. The radioactivity released into the supernatant fraction in excess of the control is given in Table 1. Urea and DOC were the most effective reagents for releasing MS2-specific RNA and host RNA. EDTA released a small amount of MS2-specific RNA, but seemed to have no effect on host components. Dithiothreitol and high salt have a negligible effect on release. High salt added to the supernatant fraction causes some precipitation of radioactivity. In other experiments not shown, low *p*H (4.0) and deoxyribonuclease were shown to have no effect. High *p*H (11.5) left considerably more ^3H-uracil on the membrane than did DOC or urea.

Approximately 50% of the radioactivity associated with the sediment is released in 0.2% DOC (Table 1). Although 90 to 95% release could be obtained in 0.5% DOC, the results reported below were obtained by using 0.2%, since Hendler and Tani (13) reported that higher concentrations alter the amount of protein associated with ribosomes. Several experiments in which 0.5% DOC was used, however, gave sedimentation patterns similar to those reported below. The remaining 5 to 10% of the RNA not released by DOC could represent a qualitatively different fraction.

Characterization of structures containing newly synthesized MS2-specific RNA. Since it is possible that the membrane may serve as a matrix for the organization of a diversity of RNA-containing structures such as "replicative complex," intermediates in viral assembly, and polyribosomes, it seemed of interest to compare the MS2-specific RNA-containing structures on the membrane with those in the supernatant fraction.

TABLE 1. *Effect of reagents upon binding of RNA to sediment*[a]

Counts/min released from sediment[b] resuspended in:	Infected (^3H %)	Infected (^{14}C %)	Uninfected (^3H %)
TMKAS + 0.2% DOC	46	67	32
8 M Urea (*p*H 9.1)	48	66	39
TMKAS + 0.01 M dithiothreitol	3	0	0
0.01 M EDTA + 0.001 M Tris (*p*H 8.0)	9	2	10
0.01 M Tris (*p*H 8.0) + 0.24 M $(NH_4)_2SO_4$	13	9	17
0.01 M Tris (*p*H 8.0) + 1.6 M $(NH_4)_2SO_4$	10	0	0
0.006 M $MgCl_2$ + 0.001 M Tris (*p*H 8.0)	0	0	0
0.01 M Tris (*p*H 8.0) + 0.24 M $(NH_4)_2SO_4$	10	7	17
0.01 M Tris (*p*H 8.0) + 1.6 M $(NH_4)_2SO_4$	0	1	23

[a] E. coli cells were labeled with ^{14}C-uracil (1.9 counts per min per ml, 0.25 μg/ml) for one doubling time and then treated with actinomycin (1 μg/ml). One-half of the culture was infected with MS2 at a multiplicity of infection of 5. At 22 min postinfection, unlabeled uracil was added to both the infected and uninfected cultures to a final concentration of 0.33 μg/ml; 2 min later, ^3H-uracil was added to 2 μc/ml. After 2.5 min, the cultures were harvested, lysed, and separated into 30,000 × g supernatant fractions and membrane fractions. The membrane fractions were suspended in TMKAS and divided into several portions which were centrifuged at 30,000 × g for 15 min. The pellets were suspended in different solutions. Each was then subjected to a final centrifugation at 30,000 × g for 15 min, and the amount of radioactivity released into the supernatant fraction was determined. The radioactivity released from a control sample suspended in TMKAS was subtracted. The release of stable RNA (^{14}C) from the uninfected culture was similar to that from the infected culture.

[b] Sediment, 100%.

The supernatant fractions and DOC-treated membrane fractions from the samples in the experiment shown in Fig. 1 were centrifuged on gradients containing no Mg^{2+}. The sedimentation patterns are shown in Fig. 2. After 1 min of labeling, a broad smear of ^3H between 10 and 40*S* was present in the membrane fraction. By 3 min of labeling, the ^3H in the membrane fraction appeared in particles with sedimentation coefficients of 27, 32, and 40*S*. For convenience, we have called these "MS2 particles," although it is

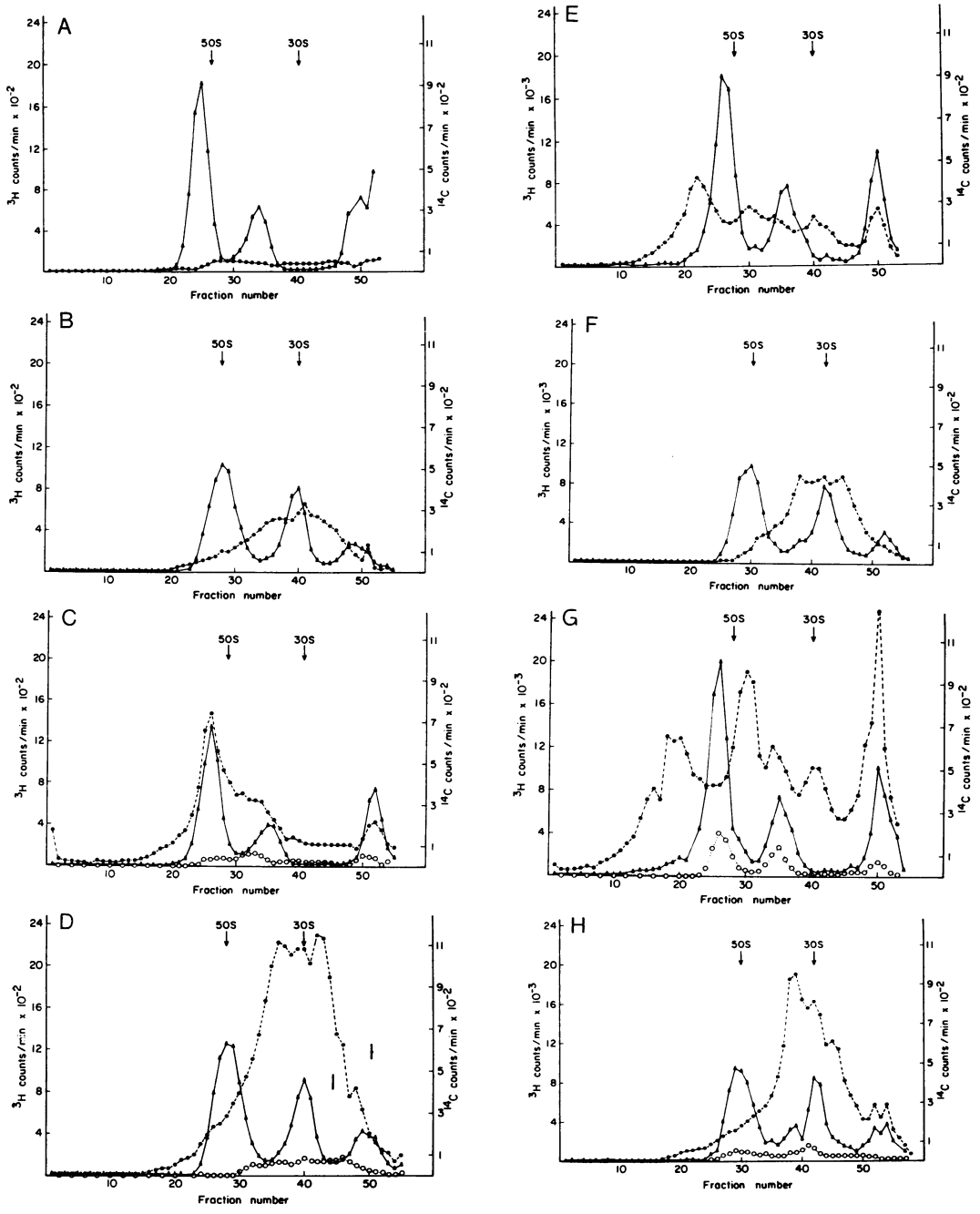

FIG. 2. *Sedimentation patterns of labeled material from supernatant and membrane fractions after different labeling periods in actinomycin. Samples (0.2 ml) of the 30,000 × g supernatant and DOC-treated membrane fractions from the experiment described in Fig. 1 were layered on linear gradients of 5 to 30% sucrose in 0.01 M Tris (pH 7.4) and 0.04 M KCl. They were centrifuged at 37,000 rev/min for 195 min in an SW41 rotor in a Spinco model L2 65B ultracentrifuge set at 5 C. Eleven-drop fractions were collected directly on filters, trichloroacetic acid-precipitated, and counted. The ^{3}H counts per minute from the uninfected culture were normalized to those in the infected culture by matching the ^{14}C counts in each. Note change of scale for ^{3}H in 8′ and 16′ sample. (A) One-minute label, supernatant fraction; (B) 1-min label, sediment; (C) 3-min label, supernatant fraction; (D) 3-min label, sediment; (E) 8-min label, supernatant fraction; (F) 8-min label, sediment; (G) 16-min label, supernatant fraction; (H) 16-min label, sediment. Symbols: ▲, ^{14}C counts per minute from infected culture (stable RNA); ●, ^{3}H counts per minute from infected culture (newly synthesized RNA); ○, ^{3}H counts per minute from uninfected culture (newly synthesized RNA).*

quite possible the 27S particle is free MS2 RNA. The samples taken after 8 and 16 min of labeling show that no new particles appear in the membrane fraction with long labeling. When material from the 40, 32, and 27S peaks was resedimented, their sedimentation coefficients had not changed. When RNA extracted from an actinomycin-treated MS2-infected culture was centrifuged with and without unlabeled cell lysate, similar patterns were obtained. Similarly, when RNA extracted from purified MS2 or ribonuclease-resistant duplexes (17) was mixed with cell lysates, their sedimentation coefficients were not altered (Cramer, *unpublished data*). Thus the MS2 particles are not due to nonspecific association of cellular protein with MS2-specific RNA.

The ^3H from the uninfected control culture is shown after 3 and 16 min of labeling. The membrane fractions show ribosomal precursor particles and ribosomal subunits. Ribosomal precursors have sedimentation coefficients of 26, 32, and 43S (19, 20). Di Girolamo et al. (4) also found ribosomal precursors mainly in a membrane fraction. This is consistent with the conclusion of Haywood (*in preparation*) that much of *E. coli* RNA first appears on membrane. The similarity of the sedimentation coefficients of the ribosomal precursors to those of the MS2 particles is presumably fortuitous. Since MS2 particles accumulate in the presence of actinomycin and since RNA bacteriophage inhibits the synthesis of 16 and 23S RNA found in ribosomal precursors (16), it does not seem likely that the MS2 particles are a result of the arrest of ribosomal maturation with accumulation of ribosomal precursors.

After 1 min of labeling in the infected culture, there is hardly any ^3H in the supernatant fraction (Fig. 2a). At 3 min (Fig. 2c), particles similar to those in the membrane fraction could not be clearly distinguished in the supernatant fraction. After 8 and 16 min of labeling, the supernatant fraction contained components that sediment at approximately 78, 62, and 46S in addition to MS2 particles. The 81S progeny virus and a component which sediments at approximately 6S appear in the supernatant fraction. The ribosomal subunits in the supernatant fraction sediment at 38 and 55S, whereas those in the membrane sediment at 30 and 50S. Since an unknown complement of ions and factors is released from the cell into the supernatant fraction, the supernatant fraction from the sample labeled at 16 min was diluted 1:10 in buffer before centrifugation (Fig. 3). Under these conditions, the ribosomal subunits sediment at 30 and 50S and the ^3H appears in 40, 32, and 27S particles but not in 78, 62 and 46S particles. The same pattern is obtained if the

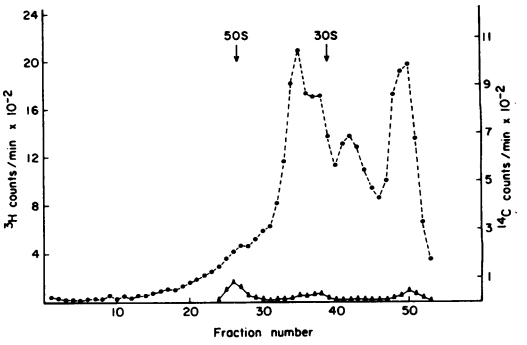

FIG. 3. *Sedimentation pattern of diluted supernatant fraction from infected samples labeled for 16 min. A 20-μliter portion of the supernatant fraction from the infected sample labeled for 16 min from the experiment described in Fig. 1 was added to 0.18 ml of TMKAS, centrifuged, and collected as described in Fig. 3. Symbols: ▲, ^{14}C counts per minute; ●, ^3H counts per minute.*

sample is diluted 1:10 in TMKAS containing either 0.005 M EDTA or 0.2% DOC. Thus it seems more likely that the 78, 62, and 46S particles represent incomplete release of the MS2 particles from polyribosomes rather than intermediates in assembly.

Hotham-Iglewski and Franklin (15) and Godson (10) showed that RNA bacteriophage RNA, including the RNA contained in a 40S particle, is found in polyribosomes. Polyribosomes are present in both the membrane and in the supernatant fractions. Thus MS2-specific RNA first appears on membrane, structures isolated in low Mg^{2+} and containing MS2-specific RNA first appear on membrane, and polyribosomes containing MS2-specific RNA are found on membrane. This suggests that organization of RNA into larger structures occurs on membrane in vivo.

Relationship between MS2 particles and virion. To investigate the relationship between the MS2 particles and the completed virus, actinomycin-treated MS2-infected and actinomycin-treated uninfected cultures were labeled for 8 min, half of each culture was harvested, and the other half was chased with an excess of cold uracil for 10 min. The radioactivity incorporated into the uninfected culture was only a small fraction of that incorporated into infected cells and shows some ribosomal precursors after an 8-min label and only ribosomal subunits after the chase. The amount of radioactivity in the infected culture after the chase was 6% greater than before the chase. The total lysates were treated with 0.2% DOC and analyzed in sucrose gradients (Fig. 4.) As previously shown in Fig. 2, after an 8-min

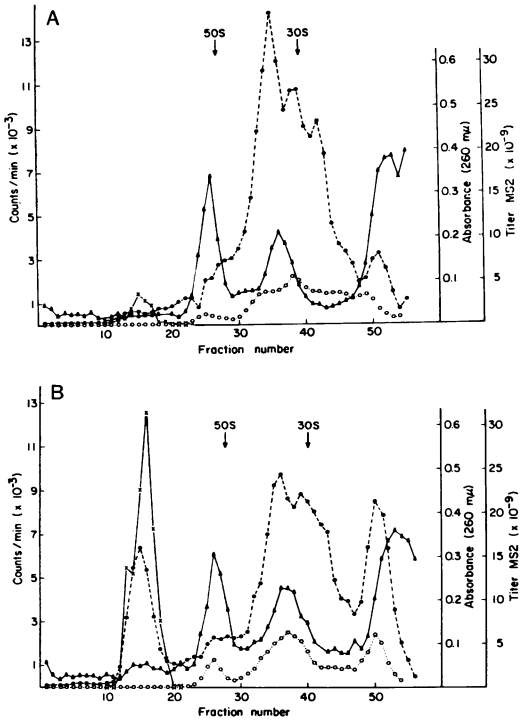

FIG. 4. *Eight-minute label and 10-min chase of MS2-infected, actinomycin-treated E. coli. E. coli cells were EDTA-treated, and actinomycin was added to a final concentration of 0.5 μg/ml. After a 10-min incubation, the culture was divided into two equal cultures, one of which was infected with MS2 (multiplicity of infection, 5). At 24 min after infection, ^{3}H-uracil and unlabeled uracil were added to both cultures to give final concentrations of 1 μc/ml and 1 μg/ml. After 8 min of labeling, both infected and uninfected cultures were divided in half (2 × 10^{10} cells), and one half was harvested. Unlabeled uracil was added immediately to the other half to a concentration of 230 μg/ml, and the cells were rapidly chilled and centrifuged. The latter two cultures were incubated for an additional 10 min after resuspension in 37 C growth medium containing 100 μg of uracil per ml, and then harvested. Since the cells were not completely converted to spherical forms by the lysozyme freeze-thaw method in this particular experiment, the tubes containing them were held in the 37 C bath for 20 sec to complete the conversion. The entire lysates were treated with 0.2% DOC. Samples (0.2 ml) of each lysate were layered on linear gradients of 5 to 30% sucrose in 0.01 M Tris (pH 7.6) and 0.04 M KCl and centrifuged at 37,000 rev/min for 3.5 hr in an SW41 rotor at 5 C. Alternately, 4 drops were collected on Whatman filters and 7 drops in tubes containing 1.1 ml of 0.01 M Tris (pH 7.6) and 0.04 M KCl. The filters were treated with cold trichloroacetic acid. The absorbance (260 nm) of the samples in tubes was read and samples were removed for MS2 titer. The amount and position of the counts in the gradients in the uninfected culture were adjusted so that they corresponded to an absorbance pattern equivalent to that in the infected culture. (A) Eight-minute pulse; (B) 8-min pulse followed by 10-min chase. Symbols ●, ^{3}H counts per minute from infected culture; ○, ^{3}H counts per minute from uninfected culture; ▲, absorbance (260 nm) infected culture; ×, MS2 titer-infected culture.*

labeling period the radioactivity in the infected culture is in the "MS2 particles." During the 10-min chase, the phage titer increased, and a peak of radioactivity coincident with the MS2 infectivity appeared in addition to a 4 to 6S peak. During the chase, there was a 30% loss of radioactivity from the 40S peak but only minor losses from the 32 and 27S peaks in the infected culture. This suggests but does not prove that the 40S particle contains the RNA that is incorporated into the virion. At this stage of the infection, it takes over 8 min to assemble a virion, and the MS2 particles do not turn over rapidly. This raises the question whether the MS2-specific RNA that does not turn over rapidly might be compartmentally and functionally distinct, e.g., be used only as messenger (or might be the result of abortive infection). Information on the location of MS2-specific proteins during and after their synthesis should help resolve this question. Such work is in progress.

Control in the absence of actinomycin D. To make certain that the observations on complexes from cells treated with actinomycin were not resulting from artifacts due to the presence of the drug, infected and uninfected cultures were labeled for 8 min in the absence of actinomycin.

Figure 5a shows sedimentation pattern of the DOC-treated membrane fraction from the uninfected culture centrifuged on a gradient containing no Mg^{2+}. The amount of label in the 30 and 50S ribosomal subunits is nearly equal. The 43S precursor of the 50S particle is considerably more prominent that it is in most experiments. This observation is consistent with the membrane fraction as the site for ribosomal precursors. Figure 5b shows the sedimentation pattern from the DOC-treated membrane fraction from the infected culture. The amount of label in the 50S subunit is much lower than that in the uninfected culture. Hudson and Paranchych (16) showed that RNA bacteriophage causes a decrease in the synthesis of ribosomal RNA. With this level of inhibition of host ribosomal synthesis, the maximum number of counts per minute that could be expected at the peak of the 30S subunit would be 400. The rest of the radioactivity in the 27 to 40S area should be in "MS2 particles." These particles correspond exactly to those found on membrane in actinomycin, except for the small con-

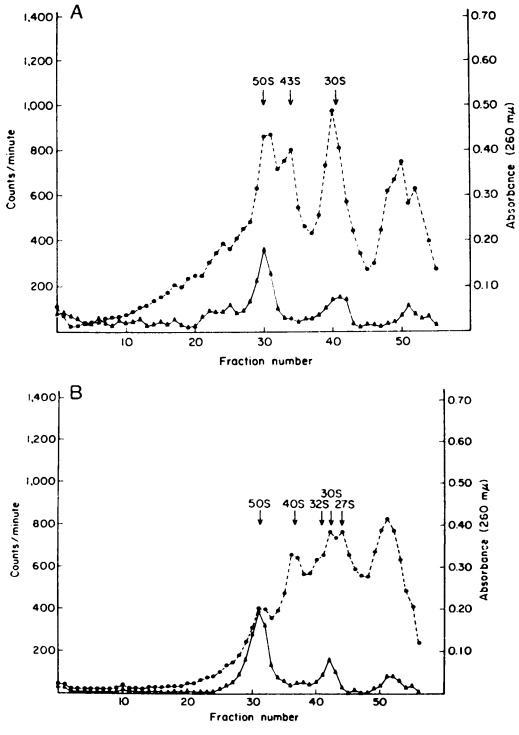

FIG. 5. *Sedimentation pattern of DOC-treated membrane fraction of MS2-infected and -uninfected E. coli labeled 8 min in the absence of actinomycin. One half of a culture of E. coli at a concentration of 3 × 10^8 cells/ml was infected with MS2 at a multiplicity of infection of 3. Eighteen minutes later, ^{3}H-uracil and unlabeled uracil were added to the infected and uninfected cultures to concentrations of 1 μc/ml and 1 μg/ml. Eight minutes after the addition of label, the cells were harvested and divided into membrane and supernatant fractions. Samples (0.15 ml) of the DOC-treated membrane fractions were layered on linear gradients of 5 to 30% (w/v) sucrose in 10 mM Tris (pH 7.6) and 40 mM KCl, and centrifuged at 37,000 rev/min for 150 min in an SW41 rotor in a Spinco model L2-50 ultracentrifuge set at 2 C. Eleven-drop fractions were collected in 1.1 ml of 10 mM Tris (pH 8) and 40 mM KCl. The pellets were resuspended in 1.1 ml of the same buffer. The absorbance (260 nm) of each fraction was read and 0.5 ml of each fraction was counted. Symbols: (A) uninfected culture; (B) MS2-infected culture; ▲, absorbance (260 nm); ●, ^{3}H counts per minute.*

tribution from the 30*S* subunit. Likewise, the association of these particles with membrane is not an artifact due to actinomycin.

DISCUSSION

Since MS2-specific RNA appears first in a structure that sediments at 30,000 × *g* for 15 min, we have postulated replicative site(s) bound to the bacterial membrane and containing both host- and MS2-specific elements. The replicative sites must contain viral polymerase and template RNA (either parental or progeny). The involvement of host components is shown by the presence in uninfected cells of factors necessary for the in vitro replication of viral RNA (5, 6) and indicated by the actinomycin sensitivity of MS2 production during the first 16 min of the growth cycle (11). Since much of host RNA is also made on a rapidly sedimenting fraction and can be released from this fraction by DOC or urea (Haywood, *in preparation*), viral use of the components of the host-RNA-synthesizing system could account for the reduction of host ribonucleate synthesis in infected cells (16).

Synthesis of nucleic acids on rapidly sedimenting structures or membranes has been reported for other viruses. Horton et al. (14) found encephalomyocarditis RNA polymerase in a fraction that sedimented between 6 × 10^3 and 10^5 *g* × min. The polymerase of foot-and-mouth-disease virus has been shown to be bound to the cell membrane (2). Poliovirus replication has been shown to occur on large membranous structures (9, 22). Most of the replicative intermediate present in Semliki Forest virus infection appears to be membrane-associated (7). Knippers and Sinsheimer (18) recently showed that the parental replicative form of the deoxyribonucleic acid (DNA) bacteriophage φX174 is associated with a rapidly sedimenting cell component.

There are examples not only of viral nucleic acid synthesis but also of host nucleic acid syntheses that occur on fast-sedimenting structures. Much of *Streptococcus faecalis* RNA (1) and *E. coli* RNA (Haywood, *in preparation*) appears on a fast-sedimenting structure. There is evidence that the growing point of *E. coli* DNA is on membrane (8, 25). Thus the association of nucleic acid synthesis with a membrane may be a general phenomenon.

In our experiments, the newly synthesized RNA is found first in 40 and 32*S* particles while still on the membrane (the 27*S* peak may well be free RNA). Histidine-containing protein was found by Haywood and Sinsheimer (12) in a 41*S* particle released from the membrane particle by DOC. Richelson and Nathans (23) have electrophoresed the polypeptides from 40*S* particles and found RNA polymerase, viral coat protein, and viral assembly protein. Details on the RNA types in the different particles will be reported later by Cramer and Sinsheimer. In the presence of high Mg^{2+}, MS2 particles can be found in polyribosomes on the membrane fraction. Thus it appears that not only synthesis but some or-

ganization of MS2-specific RNA occurs on membrane. It is not possible to absolutely eliminate the chance that MS2 RNA is transported to the membrane exceedingly rapidly for the purpose of being organized into larger structures, although it appears very unlikely, since very short pulses would be expected to show the RNA at another site under these conditions.

Further work is necessary to determine the cellular location of protein synthesis and of assembly. Furthermore, it is important to find out whether cellular compartmentalization is related to the function or fate of MS2 RNA.

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