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## Translational Control of Vesicular Stomatitis Virus Protein Synthesis: Isolation of an mRNA-Sequestering Particle

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## Translational Control of Vesicular Stomatitis Virus Protein Synthesis: Isolation of an mRNA-Sequestering Particle

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An mRNA-ribonucleoprotein particle (mRNP) was found in vesicular stomatitis virus (VSV)-infected Chinese hamster ovary cells. The particle was present 3 and 4.5 h after infection but was barely discernible at 2 h. The mRNP (buoyant density, 1.56 g/cm<sup>3</sup>), which cosedimented with viral nucleocapsid in a sucrose density gradient at approximately 120 to 160S, was separable from nucleocapsid (buoyant density, 1.31 g/cm<sup>3</sup>) by CsCl density gradient centrifugation. It contained all five VSV mRNAs and, almost exclusively, viral N protein. Some host mRNA and host protein was also present in the particle. The intact mRNP was incapable of stimulating protein synthesis in an *in vitro* protein-synthesizing system, although the VSV mRNA isolated from the particle by phenol extraction was functional *in vitro*. In contrast, intact polysomes stimulated cell-free protein synthesis to the same extent as purified polysomal mRNA. By 4.5 h after infection, 97% of the functional mRNA *in vivo* was associated with the mRNP, and only 3% was on polysomes. The amount of polysomal mRNA at 4.5 h after infection was only 31% of that found at 2 h after infection; this was reflected by the 76% decrease observed in the rate of *in vivo* protein synthesis at 4.5 h relative to that found at 2 h. Thus, it appears that the mRNP serves as an organelle which sequesters the large excess of VSV mRNA that is normally made during secondary transcription.

Vesicular stomatitis virus (VSV), a negative-stranded RNA rhabdovirus, provides an ideal system for studying the interrelationships between gene transcription and translation and between translation and messenger stability. The VSV genome codes for five proteins (12, 13, 21), and it is possible to isolate and translate the corresponding mRNAs *in vivo* (17). VSV messages can be used as a paradigm for studying some of the general properties of eucaryotic mRNA, since VSV mRNAs possess many of the features of normal eucaryotic mRNA, such as a 5' cap and a 3' polyadenylate tail (1, 2).

Soon after infection, VSV initiates the replication of the genome, its transcription into mRNA, and subsequent translation of the mRNAs (11, 27). In addition, the virus or virus-directed products inhibit host protein synthesis (19, 28). During replication, a nucleocapsid particle containing N, NS, and L proteins and a 42S genome length RNA appears (19). Recently, an *in vitro* system has been described which assembles VSV genome-size RNA plus and minus strands into nucleocapsids containing N protein (9). The presence of other VSV proteins was not fully established. Another intracellular particle containing N protein and VSV leader RNAs sedi-

menting at 18S has also been described (3). Its role in replication and transcription is not known.

The presence of mRNA-ribonucleoprotein particles (mRNPs) late in VSV infection has also been demonstrated (8, 11, 25). The particles, isolated by Grubman and Shafritz (8), were obtained from polysomes (>120S) and subribosomal particles (<80S). They are composed of two size classes, a large particle (approximately 60 to 80S) containing 26S viral mRNA and a small one (approximately 40S) containing 12 to 18S mRNA. The particles also included the VSV N protein and a host protein. In these studies, the region of 120 to 160S (the "nucleocapsid") was not analyzed, nor was quantitation of the fraction of total mRNA in these particles performed or the presence of host cell mRNA studied.

In the present communication, we characterize a new mRNP isolated from the nucleocapsid region of a sucrose density gradient which is present reasonably early (before 3 h) during VSV infection of Chinese hamster ovary cells. This particle sediments at approximately 120 to 160S and contains all five VSV mRNAs and almost exclusively the VSV N protein. Data are

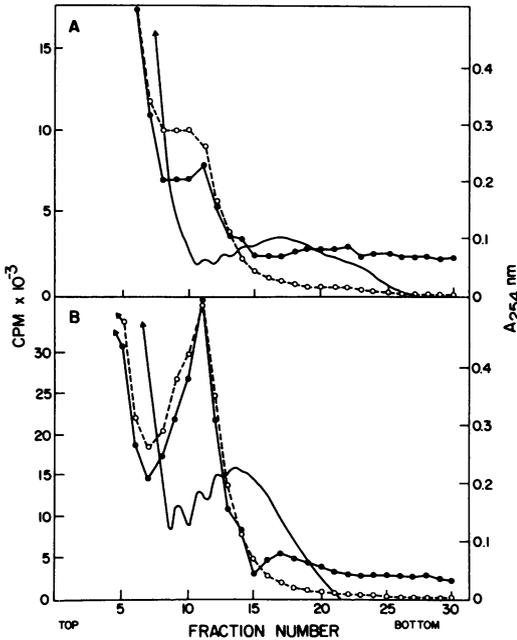


FIG. 1. mRNP at 2 (A) and 3 (B) h after VSV infection. Chinese hamster ovary cells were infected with VSV for the indicated times. The cells were labeled with [ $^{35}$ S]methionine for 10 min before the addition of puromycin. The spinner culture was then divided, and half of the cells were incubated with puromycin for 10 min. Cytoplasmic extracts were fractionated as described in the text. Radioactivity of control (●) and of puromycin (○) is shown. The solid line without symbols is the absorbance profile of polysomes before addition of puromycin.

presented which suggest that the mRNP regulates the translation of VSV protein synthesis by sequestering functional mRNA and making it inaccessible for protein synthesis.

#### MATERIALS AND METHODS

**Chinese hamster ovary cells and viral infections.** Chinese hamster ovary cells and VSV (Indiana strain) were kindly supplied by Alice Huang (Harvard Medical School, Boston, Mass.) in 1976 and have been maintained as previously described (26). All infections were carried out at 34°C in F-13 medium (GIBCO Laboratories) supplemented with 2% fetal bovine serum. Suspension cultures were infected at a multiplicity of infection of 10 in the presence of actinomycin D (10  $\mu$ g/ml) as described previously (22). Medium contained HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffers to maintain a constant pH of 7.4.

**Labeling and dissociation of polysomes.** When distribution of mRNA was used to visualize polysomes, cells were labeled continuously with [ $^3$ H]uridine (10  $\mu$ Ci/ml, 48 Ci/mmol) until completion of the experiment. When polysomal bound protein was to be used as a marker, cells were labeled with [ $^{35}$ S]methionine (20  $\mu$ Ci/ml, 1,295 Ci/mmol) 10 min before harvest.

When required, polysomes were dissociated with puromycin (300  $\mu$ g/ml) 10 min before cell harvest.

**Gradient analysis of RNA and RNP.** Cells were fractionated as described previously (20) with modifications. Cells ( $2 \times 10^6$ /ml) were centrifuged ( $500 \times g$ , 5 min), followed by two washings with Earle's balanced salt solution. Cell pellets were suspended in 1.8 ml of reticulocyte standard buffer (10 mM Tris-chloride [pH 7.2]–10 mM NaCl–1.5 mM  $MgCl_2$ ) and allowed to swell for 10 min at 0°C. Cells were then disrupted by 50 strokes in a tight-fitting Dounce homogenizer. The extracts were centrifuged ( $2,000 \times g$ , 4 min) to remove nuclei. Postnuclear supernatants were made 1% in Nonidet P-40 to dissolve membranes. The supernatants were layered onto 36-ml, 5 to 40% (wt/vol) sucrose gradients in high-salt buffer (10 mM Tris-chloride [pH 7.2]–500 mM NaCl–50 mM  $MgCl_2$ ). Centrifugation was carried out at 3°C for 2.5 h at 25,000 rpm with a Spinco SW27 rotor. Gradients were fractionated with an ISCO density gradient fractionator, (Instrumentation Specialties Co.) and the UV absorbance at 254 nm was monitored. Identification of polysome and mRNP fractions was accomplished by removing 50- $\mu$ l samples from each gradient fraction of separate gradients of extracts of puromycin-treated or untreated infected cells and measuring the amount of trichloroacetic acid-precipitable radioactivity (usually in the form of [ $^{35}$ S]methionine-labeled protein). The extract from puromycin-treated cells gave the mRNP, and in the untreated control, the polysome fractions past the mRNP were collected. When required, the mRNP was further purified from comigrating polysomes by pooling mRNP fractions taken directly from the gradient, dialyzing against reticulocyte standard buffer for 12 h, and separating the dialyzed material on CsCl gradients (Fig. 2).

**Extraction of RNA.** RNA was isolated from the polysomal and mRNP fractions as previously described (7, 24), washed in 70% ethanol, and stored at -70°C in a small volume of sterile water until further use. In experiments involving mRNA bound to intact particles, recovery of purified mRNP was accomplished by centrifugation in a Beckman 65 rotor at 45,000 rpm for 4.5 h through 32% sucrose in high-salt buffer. Polysomal RNA still associated with polysomes was obtained in the same way.

**Cell-free protein synthesis.** Seven micrograms of total RNA per 50- $\mu$ l reaction sample was used to direct an in vitro cell-free wheat germ protein-synthesizing system or a rabbit reticulocyte lysate as described previously (22, 23). Incorporation of RNA at this concentration was in the linear range.

**Gel electrophoresis of protein, peptide analysis, and fluorography.** Both in vivo and in vitro protein products, as measured by incorporation of [ $^{35}$ S]methionine, were analyzed on 10% polyacrylamide slab gels as described previously (7). Peptide mapping of the mRNP and the viral N protein was done by the method of Cleveland et al. (5) with *Staphylococcus* V8 protease (Miles Laboratories). Dried gels were fluorographed by the procedure of Laskey and Mills (14).

#### RESULTS

**Subcellular localization of VSV mRNA and protein.** The subcellular localization of VSV RNA and protein was determined. Cells were

labeled with either [ $^{35}\text{S}$ ]methionine for 10 min at 2 and 3 h after VSV infection or [ $^3\text{H}$ ]uridine continuously for 2 and 3 h before cell harvest. At each time, half of the spinner culture was incubated with puromycin (300  $\mu\text{g}/\text{ml}$ ) for 10 min to dissociate active polysomes (10). In this way, it was possible to distinguish between polysome-bound mRNA and RNA which sedimented with polysomes but was not functionally bound to them. At both 2 and 3 h (Fig. 1A and B), fractions 15 to 30 were sensitive to the drug, as evidenced by polysome breakdown (data not shown). It is noteworthy, however, that there was a puromycin-resistant, protein-containing particle (mRNP) (Fig. 1B) which encompassed the entire small polysomal region of the gradient (approximately 120 to 160S). The same results were obtained when viral RNA was labeled with [ $^3\text{H}$ ]uridine (data not shown). Consequently, the particle contains both protein and RNA. The mRNP also remained intact when cytoplasmic extracts were analyzed on sucrose gradients containing 20 mM EDTA, which also dissociated polysomes (data not shown).

The mRNP was purified by CsCl gradient sedimentation. VSV-infected cells were labeled continuously with [ $^3\text{H}$ ]uridine and then labeled with [ $^{35}\text{S}$ ]methionine for 15 min before harvesting the cells. In this way, both viral RNA and protein were labeled. Cytoplasmic extracts were prepared, and the crude particles were collected from the appropriate fractions of a sucrose density gradient. The mRNP labeled in protein with [ $^{35}\text{S}$ ]methionine and in RNA with [ $^3\text{H}$ ]uridine was separated from the nucleocapsid by CsCl density gradient centrifugation of crude mRNP fractions. The buoyant density of the mRNP was 1.56  $\text{g}/\text{cm}^3$ , and that of the nucleocapsid was 1.31  $\text{g}/\text{cm}^3$ , a value reported by others (11; Fig. 2). It is noteworthy that most of the protein (69% of total) was associated with the nucleocapsid, and practically all of the mRNA (92%) was found in the mRNP. The mRNPs were not fixed before sedimentation through CsCl, yet they seemed quite stable. Less than 10% of the total mRNA put on the gradient was found in the pellet, and no protein was present in the top of the gradient, where it would be expected if both macromolecules were stripped off the particles.

**Characterization of the RNA and protein present in the mRNP.** The presence of VSV mRNA in the mRNP was demonstrated by its *in vitro* translation. Cells infected for 3 h were treated with puromycin to disaggregate the polysomes. Cytoplasmic extracts were made and separated by density gradient centrifugation, and the fractions containing the mRNP were collected by centrifugation through 32% sucrose made up in high-salt buffer. The respective particles were separated by centrifugation through CsCl. RNA

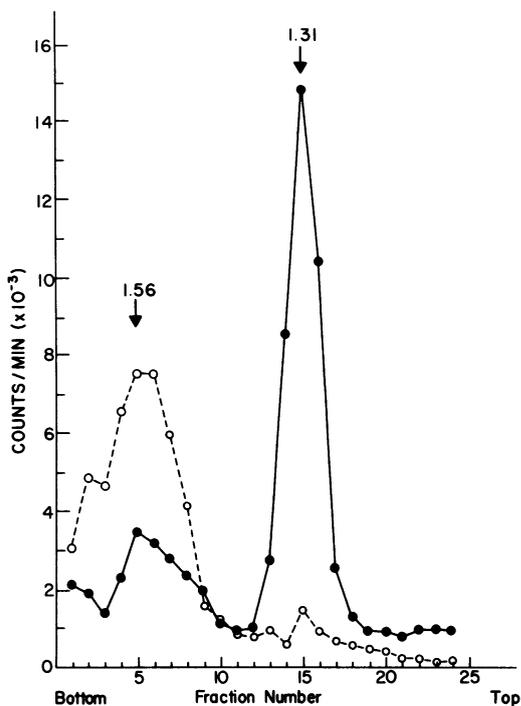


FIG. 2. CsCl density gradient centrifugation of mRNP fraction. Cells were infected for 3.5 h. Cytoplasmic extracts were prepared and analyzed on sucrose density gradients as described in the text. The mRNP-containing fractions were dialyzed extensively against reticulocyte standard buffer and then lyophilized. The material sedimenting within this fraction was centrifuged on a 5-ml preformed CsCl gradient (1.17 to 1.65  $\text{g}/\text{cm}^3$ ) at 35,000 rpm for 10 h in a Spinco SW50.1 rotor. [ $^{35}\text{S}$ ]methionine incorporation during a 10-min pulse (●) and [ $^3\text{H}$ ]uridine incorporation after labeling for 3.5 h (○) is shown.

from each particle was isolated by phenol extraction and used to program an *in vitro* cell-free reticulocyte protein-synthesizing system. The [ $^{35}\text{S}$ ]methionine-labeled proteins synthesized were identified by gel analysis. It was clear (Fig. 3A, lane 3) that the particle contained mRNA which coded for the G, N, NS, and M VSV proteins. L protein was not made *in vitro* (8, 11), but it was observed in a gradient of RNA as 28S. The mRNP did not contain rRNA (data not shown) and was therefore not contaminated with polysomes which have the same buoyant density. Some host protein was made *in vitro* (Fig. 3A, lane 3), indicating that some host mRNA was present in the particle. Although prominent in the overexposed gel, the presumed host proteins made up a small portion of the total translation products. One prominent protein (R in 3A) was an endogenous reticulocyte protein made in extracts lacking added mRNA. Both the R protein and another one running slightly slower than

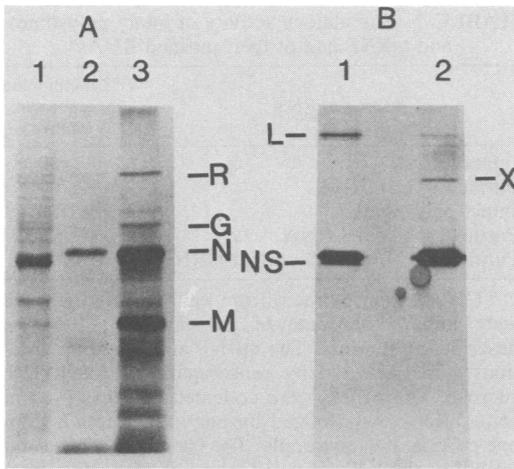


FIG. 3. Polyacrylamide gel analysis of mRNP- and nucleocapsid-associated proteins and in vitro synthesized proteins with particle mRNA as template. Particles were isolated from cells infected with VSV for 3 h, collected from sucrose density gradients, and dialyzed to get rid of the sucrose. The particles were separated on a CsCl gradient as described in the legend to Fig. 2 and dialyzed to get rid of the CsCl. (A) RNA from each particle (60  $\mu$ g of protein) was purified by phenol treatment and suspended in 10  $\mu$ l of water. Three microliters of each RNA sample was added to a reticulocyte lysate, and the translation products were separated on a polyacrylamide gel. Lane 1, Extract of uninfected cells labeled for 15 min with [<sup>35</sup>S]methionine (35,000 cpm); lane 2, [<sup>35</sup>S]methionine-labeled translation products with RNA from the 1.31-g/cm<sup>3</sup> particle (25,000 cpm); lane 3, translation products from 1.56-g/cm<sup>3</sup> particle (96,000 cpm). (B) As described in the text, VSV cells infected for 3 h were labeled with [<sup>35</sup>S]methionine for 15 min. The proteins from the respective particles were run on a 10% polyacrylamide gel. Lane 1, 1.31-g/cm<sup>3</sup> particle (18,000 cpm); lane 2, 1.56-g/cm<sup>3</sup> particle (18,500 cpm). A reaction with no added mRNA gave the same pattern as that shown in (A), lane 2. Abbreviations: L, G, N, NS, and M are the respective VSV proteins; R, reticulocyte protein made in the translation system lacking added mRNA; X, unknown host protein.

VSV N protein in translations of RNA extracted from the nucleocapsid were endogenous reticulocyte lysate proteins made in the absence of added mRNA (Fig. 3A, lane 2). Therefore, the nucleocapsid apparently has no mRNA associated with it. However, some 42S genome RNA was present (although plus- or minus-strand RNA was not determined), as shown by sucrose density gradient analysis (data not shown). Viral genome has been previously shown to be present in the nucleocapsid (11). No 42S RNA was found in the 1.56-g/cm<sup>3</sup> particle.

To determine the protein composition of the mRNP and nucleocapsid, cells infected for 3 h

were labeled with [<sup>35</sup>S]methionine 10 min before harvesting. The mRNP and nucleocapsid were collected, and the labeled protein was analyzed by polyacrylamide gel electrophoresis. The mRNP contained, almost exclusively, a protein of the same molecular weight as VSV N protein (Fig. 3B, lane 2) and a trace of L protein. In addition, a 90,000-dalton protein, presumably a host protein, was also observed (see "X" in Fig. 3B). Peptide maps of both authentic N protein and the protein present in the particle proved that both proteins were identical (Fig. 4).

In contrast to what was observed in mRNP, the nucleocapsid (Fig. 3B, lane 1) contained L and NS protein in addition to the major N protein.

**Distribution of viral mRNA between polysomes and mRNP.** The distribution of functional viral mRNAs between polysomes and mRNP was determined by translating purified RNA from the respective fractions in vitro. Stimulation of incorporation by each sample of RNAs was taken as a measure of the amount of functional messenger present (Table 1). There was an approximately fivefold increase in total functional mRNA (polysomes plus RNP) from 2 to 3 h postinfection and a further twofold increase from 3 to 4.5 h postinfection, although there was a slightly less than twofold increase in polysome-bound mRNA from 2 to 3 h and a fivefold decrease from 3 to 4.5 h. The vast majority of the mRNA present at 3 h (75%) and at 4.5 h

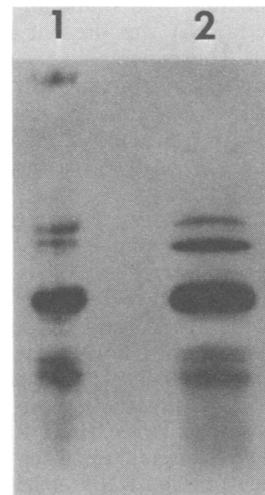


FIG. 4. Peptide analysis of mRNP protein. The peptides released by *Staphylococcus* V8 protease were analyzed on a 15% sodium dodecyl sulfate-polyacrylamide gel as described previously (5). Lane 1, Authentic VSV N protein; lane 2, protein isolated from mRNP. The proteins were isolated from 10% gels.

TABLE 1. Distribution of viral mRNA between mRNP and polysomes<sup>a</sup>

Sample and h postinfection	[ <sup>35</sup> S]methionine incorporated (cpm)
<b>mRNP</b>	
2	3,404
3	79,098
4.5	188,688
<b>Polysomes</b>	
2	17,325
3	26,500
4.5	5,429

<sup>a</sup> Polysomal and mRNP fractions were separated from cytoplasmic extracts of infected cells on sucrose density gradients as described in the text. RNA was isolated from each fraction by phenol extraction. The RNA content of each fraction before and after phenol treatment was determined by the orcinol method. In this way, the percent recovery of RNA in each fraction was determined. It was thus possible to add amounts of purified RNA to each 5- $\mu$ l wheat germ cell-free protein-synthesizing reaction in proportion to the amount initially present in the sucrose gradient fractions. Therefore, the data presented quantitatively show the mRNA content in each fraction. Total incorporation was proportional to the amount of RNA added and is therefore a reflection of the mRNA content of the samples. At 2 h, 84% of the total mRNA sedimenting at 80S or greater was present in polysomes, and 16% was present in the mRNP region of the gradients; at 3 h, the values were 25% for polysomes and 75% for mRNP; and at 4.5 h, the values were 3 and 97%, respectively.

(97%) postinfection was found in the mRNP, whereas little mRNA was present at 2 h in the mRNP (16%). These percentages refer to the fraction of the VSV mRNA present in the polysome region of the gradient, and not to anything less than 80S. At 2, 3, and 4.5 h, about 35, 80, and 65% of the VSV mRNA was on polysomes, respectively (18).

The *in vitro* stimulatory activity of the intact mRNP was also determined. The mRNP was isolated from cells infected for 3 h by centrifugation through a solution of 32% sucrose in high-salt buffer of the appropriate gradient fractions of a cytoplasmic extract. Polysomes were isolated in the same way. The pellets were dissolved in water and added directly to an *in vitro* wheat germ protein-synthesizing extract. RNA isolated from each of the samples was also translated *in vitro*. Although purified RNA isolated from the mRNP was very active in stimulating protein synthesis (77,709 cpm), the intact mRNP was not (400 cpm) (Table 2). Furthermore, the intact mRNP at this concentration did not significantly inhibit protein synthesis directed by purified mRNP RNA. In addition, intact polysomes and polysomal RNA were about equally effective in

TABLE 2. Stimulatory activity of intact polysomes and mRNP and of their purified RNAs<sup>a</sup>

Source of RNA	[ <sup>35</sup> S]methionine incorporated (cpm)
Intact mRNP	400
Purified mRNP RNA	77,709
Intact polysomes	11,100
Purified polysomal RNA	15,000
Purified mRNP RNA + intact mRNP	58,500

<sup>a</sup> Cytoplasmic extracts from cells infected for 3 h were prepared and analyzed on sucrose gradients as described in the text. The mRNP and polysome fractions were collected by centrifugation through 32% sucrose. The pellets were collected and dissolved in water. RNA was isolated by phenol extraction from one portion of each sample. The intact polysomes and mRNP or the RNA isolated from the respective fractions were used to program an *in vitro* wheat germ protein-synthesizing system. Seven micrograms of each RNA was used in a 50- $\mu$ l reaction.

stimulating incorporation *in vitro* (11,110 and 15,000 cpm, respectively).

**Correlation between protein synthesis and distribution of mRNA on polysomes and mRNP during VSV infection.** Previous work has shown that both the rate of protein synthesis and polysome content decrease late in VSV infection (4, 11, 15). We determined whether the decrease in protein synthesis was accompanied by a corresponding decrease in the amount of polysome-bound mRNA. Polysomes and mRNP were isolated from cells at 2, 3, and 4.5 h after infection, and their mRNA content was determined. The relative rates of *in vivo* protein synthesis correlated very well with the polysomal mRNA content at each time of infection (cf. rate of protein synthesis and polysomal mRNA content in Table 3). For example, at 4.5 h after infection, there was more than twice as much mRNA in

TABLE 3. Relative rates of protein synthesis, polysome content, polysomal mRNA, and mRNP content during VSV infection

Time after infection (h)	Rate of protein synthesis <sup>a</sup>	Content of <sup>b</sup> :		
		Polysome <sup>c</sup>	Polysomal mRNA <sup>d</sup>	mRNP-mRNA <sup>d</sup>
2	1.00 <sup>b</sup>	1.00	1.00	1.00
3	1.04	1.24	1.53	23.24
4.5	0.24	0.52	0.31	55.43

<sup>a</sup> Measured by a 10-min pulse with [<sup>35</sup>S]methionine (see text).

<sup>b</sup> Two-hour values were taken as 1.00; 3- and 4.5-h values were calculated relative to 2 h.

<sup>c</sup> Measured as area in the polysome region of sucrose gradients.

<sup>d</sup> Measured by cell-free protein synthesis (data from Table 1).

the mRNP than at 3 h, with only one-fifth as much mRNA in polysomes (Tables 1 and 3).

In contrast, the mRNA content of the mRNP was not correlated to the rate of protein synthesis, rising dramatically during the same period (see mRNP-mRNA content in Table 3). For example, the mRNA content of the mRNP at 4.5 h after infection was 55-fold that at 2 h, yet the rate of protein synthesis actually decreased to one-third the 2-h rate.

### DISCUSSION

In the present investigation, we report on an mRNP found in VSV-infected cells. The particle was present in the nucleocapsid region of the sucrose density gradients in the 120 to 160S region (Fig. 1), could be separated from the nucleocapsid (buoyant density, 1.31 g/cm<sup>3</sup>) by CsCl density gradient sedimentation, and had a buoyant density of 1.56 g/cm<sup>3</sup> (Fig. 2). The mRNP contained all five VSV mRNAs and a small amount of host mRNA (Fig. 3), but no ribosomal RNA. This particle also included almost exclusively viral N protein (Fig. 3). A 90,000-dalton protein (presumably host) is also present, but at less than 0.1% the concentration of N.

As previously described (9), the nucleocapsid contains L, N, and NS proteins. It does not possess host mRNA (Fig. 3).

Unlike the mRNP which we isolated from the nucleocapsid region of a sucrose gradient (120 to 160S), mRNPs studied by others (8) were isolated only from polysomes (>120S) or subribosomal particles (<80S). These particles contain viral N protein in addition to a 78,000-dalton host protein but sediment at approximately 60 and 40S (buoyant density, 1.36 g/cm<sup>3</sup>).

Most important, we have shown that the mRNA sequestered in the mRNP is nonfunctional *in vitro*. The intact particle and purified mRNA isolated from the particle were assayed for stimulatory activity in a wheat germ protein-synthesizing system. The purified mRNA stimulated very well, whereas the intact particle did not (Table 2). In addition, the particle itself was not inhibitory, because it did not inhibit protein synthesis when added to a wheat germ extract simultaneously with VSV mRNA. In contrast, mRNA bound to polysomes was functional *in vitro* (Table 2). Therefore, the inability of the mRNP-bound mRNA to function *in vitro* is due to some intrinsic property of the particle. It is tempting to extrapolate from this finding that particle-bound mRNA in the intact cell is likewise nonfunctional.

We previously reported that a large proportion of what was presumably polysome-bound VSV mRNA made between 2 and 3 h after infection is not translated *in vivo* (18). In these

studies, the entire polysome region displayed on a sucrose gradient was collected, and the RNA was isolated. Estimates of functional mRNA were made by determining the stimulatory activity of the RNA in a wheat germ protein-synthesizing system. It was assumed that all of the mRNA which was contained within the polysomal region of the gradient was actually bound to polysomes. In retrospect, it is obvious that this is not so. As we have shown (Table 1), 75% of the VSV mRNA at 3 h is sequestered in the mRNP, which is presumably inactive *in vivo*. Only 16% of the total VSV mRNA is found in an mRNP at 2 h postinfection. We estimate that the amount of mRNA actually bound to polysomes *in vivo* is sufficient to support the amount of VSV protein synthesis observed at either time of infection. Therefore, the control over the translation of VSV protein synthesis during infection is obtained by preventing the majority of the mRNA from binding to polysomes and acting as templates for VSV protein synthesis.

In addition, it is known that both the rate of protein synthesis and polysome content decrease late in infection (4, 11, 15). This decrease in protein synthesis is accompanied by a decreased amount of mRNA on polysomes, even though the total mRNA content of the cell increases throughout infection. The data presented in Table 3 intimate, as also discussed in the previous paragraph, that the amount of mRNA on polysomes accurately reflects the protein-synthesizing capacity of the cell, whereas the total mRNA content (polysomes plus mRNP) does not. The mRNA bound to the mRNP is unavailable for protein synthesis. It has recently been reported that cell-free extracts of VSV-infected L cells lose their ability to synthesize VSV proteins programmed by VSV mRNAs. However, addition of eucaryotic initiation factor 2 restores the protein-synthesizing capacity of the extracts (4). It is probable that these extracts contain large amounts of the mRNP we have described. If so, it is possible that the mRNA-sequestering ability of the mRNP and the loss of functional eucaryotic initiation factor 2 *in vivo* are related. We are now studying this possibility.

Host protein synthesis is inhibited during VSV infection (19, 28). However, the mRNAs for these proteins are still present late in infection and can be translated *in vitro* (6). One mechanism advanced to explain this observation is that the large excess of VSV mRNA made during infection effectively outcompetes for ribosomes and thereby prevents host mRNA from being translated (15, 16). We feel that this explanation should be reexamined, since we find the vast majority of VSV mRNA is unavailable for

translation and is therefore presumably unable to compete for sites on polysomes.

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