Greene, L. E., Chalovich, J., & Eisenberg, E. (1982) *Biophys. J. 37*, 265a.
Harrington, W. F. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5066–5070.
Huxley, A. F., & Simmons, R. M. (1971) *Nature (London) 233*, 533–538.
Huxley, H. E. (1969) *Science (Washington, D.C.) 164*, 1355–1356.
Huxley, H. E., Simmons, R. M., Faruqi, A. R., Kress, M., Bordas, J., & Koch, M. H. J. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2297–2301.
Huxley, H. E., Simmons, R. M., Faruqi, A. R., Kress, M., Bordas, J., & Koch, M. H. J. (1983) *J. Mol. Biol. 169*, 469–506.
Laemmli, U. K. (1970) *Nature (London) 277*, 680–685.
Lovell, S. J., & Harrington, W. F. (1981) *J. Mol. Biol. 149*, 659–674.
Lovell, S. J., Knight, P. J., & Harrington, W. F. (1981) *Nature (London) 293*, 660–664.
Marston, S. B. (1973) *Biochim. Biophys. Acta 305*, 397–412.
Marston, S. B., Rodger, C. D., & Tregear, R. T. (1976) *J. Mol. Biol. 107*, 263–276.
Marston, S. B., Tregear, R. T., Rodger, C. D., & Clarke, K. L. (1979) *J. Mol. Biol. 128*, 111–126.
Mornet, D., Pantel, P., Audemard, E., & Kassab, R. (1979) *Biochem. Biophys. Res. Commun. 89*, 925–932.
Nihei, T., Mendelson, R. A., & Botts, J. (1974) *Proc. Natl. Acad. Sci. U.S.A. 73*, 302–306.
Reedy, M. C., Reedy, M. K., & Goody, R. S. (1983) *J. Muscle Res. Cell Motil. 4*, 55–81.
Reedy, M. K. (1967) *Am. Zool. 7*, 465–481.
Reedy, M. K., Holmes, K. C., & Tregear, R. T. (1965) *Nature (London) 207*, 1276–1280.
Reisler, E., & Liu, J. (1982) *J. Mol. Biol. 157*, 659–669.
Reisler, E., Liu, J., & Cheung, P. (1983) *Biochemistry 22*, 4954–4960.
Rodger, C. D., & Tregear, R. T. (1973) *J. Mol. Biol. 86*, 495–497.
Rome, E. (1967) *J. Mol. Biol. 27*, 591–602.
Schaub, M. C., & Watterson, J. G. (1981) *Biochimie 63*, 291–303.
Schaub, M. C., Watterson, J. G., Pfister, M., & Waser, P. G. (1975) in *Calcium Transport in Contraction and Secretion* (Carafoli, E., Clementi, F., Drabikowski, W., & Margreth, A., Eds.) pp 479–488, North-Holland Publishing Co., Amsterdam.
Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Sutoh, K., & Harrington, W. F. (1977) *Biochemistry 16*, 2441–2449.
Thomas, D. D., & Cooke, R. (1980) *Biophys. J. 32*, 891–906.
Trayer, H. R., & Trayer, I. P. (1983) *Eur. J. Biochem. 135*, 47–59.
Ueno, H., & Harrington, W. F. (1981) *J. Mol. Biol. 149*, 619–640.
Ueno, H., & Harrington, W. F. (1984) *J. Mol. Biol. 173*, 35–61.
Weeds, A. G., & Pope, B. (1977) *J. Mol. Biol. 111*, 129–157.
Yamamoto, K., & Sekine, T. (1979) *J. Biochem. (Tokyo) 86*, 1855–1862.
Yanagida, T. (1981) *J. Mol. Biol. 146*, 539–560.

Inhibition of Protein Synthesis in Vesicular Stomatitis Virus Infected Chinese Hamster Ovary Cells: Role of Virus mRNA-Ribonucleoprotein Particle†

Craig A. Rosen,‡ John Siekierka, Herbert L. Ennis,* and Paul S. Cohen

ABSTRACT: Although host protein synthesis is preferentially inhibited, there is a steady decline in the ability of Chinese hamster ovary (CHO) cells infected with vesicular stomatitis virus (VSV) to synthesize both host and viral proteins. We previously reported finding an mRNA-ribonucleoprotein particle (mRNP) that contained all five VSV mRNAs and viral N protein exclusively. This particle apparently regulates translation by sequestering a majority of the VSV mRNA made late in infection and thus rendering it unavailable for protein synthesis. In the present investigation the mRNP was also shown to inhibit in vitro protein synthesis in rabbit reticulocyte and wheat germ lysates programmed with mRNA isolated from VSV-infected cells. The synthesis of the eIF-2·GTP·Met-tRNA (ternary) complex, the first step in initiation of protein synthesis, was markedly inhibited by the mRNP. The inhibition was partially reversed by addition of purified eIF-2 to the inhibited lysate or ternary complex formation reaction. These results indicate a dual role of the mRNP in regulating protein synthesis during infection. Nucleocapsid also inhibited in vitro protein synthesis, although this inhibition was not reversed by eIF-2. Nucleocapsid did not inhibit ternary complex formation in vitro. Consequently, nucleocapsid may also regulate in vivo protein synthesis, but by a mechanism different from the mRNP.

Early during infection of cells by vesicular stomatitis virus (VSV) host protein synthesis is selectively inhibited, and viral proteins are made (Mudd & Summers, 1970; McAllister & Wagner, 1976; Wertz & Youngner, 1972; Wagner, 1975). Later in infection, both host and viral protein syntheses are markedly inhibited. At all times both host and VSV mRNAs are competent for translation in in vitro protein synthesis systems (Lodish & Porter, 1980; Ehrenfeld & Lund, 1977).

The mechanism by which VSV selectively inhibits host protein synthesis has not been fully elucidated. Nuss et al. (1975) argue that VSV mRNAs initiate protein synthesis

† From the Department of Microbiology, University of Rhode Island, Kingston, Rhode Island 02881 (C.A.R. and P.S.C.), and Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110 (J.S. and H.L.E.). *Received October 20, 1983.*
‡ Present address: Dana-Farber Cancer Institute, Boston, MA 02115.

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better than host mRNAs, and thus VSV protein synthesis is favored. Lodish & Porter (1980, 1981) claim that the large amount of VSV mRNA that is made during infection effectively outcompetes host mRNA for a constant limited number of ribosomes. In contrast to this later finding, recent work (Schnitzlein et al., 1983; Dunigan & Lucas-Lenard, 1983) indicates that inhibition of host protein synthesis is not dependent on the intracellular concentration of VSV mRNA. Stanners et al. (1977) suggest that a virus function, called P, is involved in host protein synthesis inhibition.

However, a reasonable amount of data is available that points to initiation of translation as the site in the cell at which protein synthesis is inhibited (Nuss et al., 1975; Stanners et al., 1977; Jaye et al., 1982; Centrella & Lucas-Lenard, 1982; Gillies & Stollar, 1982; Thomas & Wagner, 1983). Centrella & Lucas-Lenard (1982) have demonstrated that eIF-2 is inactivated after VSV infection. Inactive extracts of infected cells can be stimulated to synthesize protein by addition of a partially purified preparation of eIF-2. A recent report (Thomas & Wagner, 1983), in contrast, states that the factor inactivated might be ribosome-associated proteins that coprecipitate from extracts at the same ammonium sulfate concentration as eIF-3 and eIF-4B. One report has suggested that a double-stranded RNA produced during infection can inhibit cell free protein synthesis, but its relation to in vivo events is not clear (Thomas & Wagner, 1982).

What occurs during infection that results in the loss of initiation factor activity? We recently described an mRNA-ribonucleoprotein particle (mRNP) isolated from VSV-infected Chinese hamster ovary (CHO) cells which accumulates late in infection (Rosen et al., 1982). The mRNP (buoyant density 1.56 g/cm^3), which cosediments with viral nucleocapsid (buoyant density 1.31 g/cm^3) in sucrose density gradients, is separable from the nucleocapsid by CsCl density gradient centrifugation. The mRNP contains all five VSV mRNAs and exclusively viral N protein. By 4.5 h after infection, the majority of the VSV mRNA is sequestered in the particle and is presumably unavailable to participate in protein synthesis. This could account for the observation that more VSV mRNA is made during infection than is translated (Lynch et al., 1981).

In the present investigation, we show that the mRNP can also inhibit in vitro protein synthesis. This is apparently accomplished by a sequestration or inactivation of eIF-2. Consequently, the large quantity of particle found late in infection might be responsible for inhibition of total protein synthesis observed late in infection.

Materials and Methods

Growth of Cells, Infection, and Preparation of mRNP and Nucleocapsid. CHO cells were grown in suspension culture in F-13 medium (Grand Island Biological, Grand Island, NY) supplemented with 2% fetal bovine serum. Cell maintenance and viral infections are described elsewhere (Pennica et al., 1979; Stampfer et al., 1969). Viral mRNP particles were obtained as follows: CHO cells were infected for 3 h at 34 °C and incubated with puromycin (250 μg/mL) for 10 min prior to cell harvest. Cells were sedimented (2000g × 10 min) and washed 2 times with ice-cold Earle's saline. Approximately 2 × 10^8 cells were resuspended in 2 mL of reticulocyte standard buffer [RSB, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.2, 10 mM NaCl, and 1.5 mM $MgCl_2$] and allowed to swell for 10 min at 0 °C. Cytoplasmic extracts were prepared and analyzed by sucrose density centrifugation in high salt buffer as previously described (Rosen et al., 1982). Gradients were fractionated by using a Model 640 density gradient fractionator (ISCO Labs, Lincoln, NB), and the 120–160S region of the sucrose gradient was collected. This fraction was dialyzed against sterile distilled water, and 0.5 mL was layered onto a 4.5-mL CsCl gradient of density 1.17–1.65 g/cm^3 in RSB buffer containing 1% Nonidet NP-40. Centrifugation was at 35 000 rpm for 9.5 h at 4 °C in a Beckman-Spinco SW 50.1 rotor. Gradients were fractionated, and the refractive index was monitored on a Bausch & Lomb refractometer. Fractions containing the nucleocapsid (density 1.31 g/cm^3) and mRNP (density 1.56 g/cm^3) were dialyzed against sterile distilled water and frozen in small aliquots at −70 °C. [^{35}S]Methionine-labeled particles were prepared as described above except cells were labeled with [^{35}S]methionine (50 μCi/mL, 20 Ci/mmol; Amersham, Arlington Heights, IL) for 20 min prior to addition of puromycin. Uridine-labeled particles were prepared from infections carried out in the presence of [^{3}H]uridine (10 μCi/mL, 45 Ci/mmol; New England Nuclear corp., Boston, MA) and actinomycin D (5 μg/mL) as described elsewhere (Lynch et al., 1981).

Preparation of VSV RNA. VSV RNA (from cells infected for 4.5 h) used for in vitro translation assay was ethanol precipitated from the polysome region of sucrose gradients and phenol purified according to the method of Rose & Knipe (1975). Reactions were carried out in reticulocyte lysates or wheat germ extracts as previously described (Roberts & Patternson, 1973; Pelham & Jackson, 1976). The experimental procedure is described in the legends to the figures.

Gel Electrophoresis and Fluorography. Electrophoresis of protein on 10% sodium dodecyl sulfate (SDS)–polyacrylamide gels was according to Laemmli (1970). Fluorography of dried gels was done according to the method of Bonner & Laskey (1974). All protein determinations were done according to the method of Lowry et al. (1951).

Results

Further Characterization of mRNP. We previously showed that the mRNP and nucleocapsid differ with respect to mRNA and protein content (Rosen et al., 1982). The structures of the mRNP and nucleocapsid were examined by electron microscopy and were found to be different both in size and in structure (data not shown). No ribosomes were visible in either of the particle preparations.

The question of whether the mRNP exists in vivo or is an artifact of the preparation procedure was investigated. RNA and protein can be cross-linked in vivo by UV light when they are in close association (Greenberg, 1979; Wagenmakers et al., 1980). The RNA–protein complex thus formed becomes resistant to phenol extraction. Actinomycin D treated CHO cells were infected with VSV for 3 h at 34 °C in the presence of [^{3}H]uridine. This procedure specifically labels VSV mRNA, as host RNA synthesis is inhibited by the drug. One portion of the infected cells was irradiated as described in Table I, and the mRNP from these cells and from the unirradiated cells were purified by CsCl density gradient centrifugation. Each fraction was extracted with phenol, and the amount of tritium extracted in the aqueous and phenol phases was determined (Table I). Approximately 68% of the [^{3}H]RNA was extracted into the aqueous phase of the unirradiated control, whereas only 18% was present in the same fraction from irradiated infected cells. The results therefore suggest that the mRNP is present in vivo.

Inhibition of Total Protein Synthesis during Infection. VSV infection leads to an eventual inhibition of total protein synthesis (McAllister & Wagner, 1976; Mudd & Summers, 1970; Wertz & Youngner, 1972). The data presented in Figure 1 substantiated this finding. In this experiment, infected and uninfected cells were labeled for 1-h periods, and the amount

Table I: In Vivo Cross-Linking of ^{3}H-Labeled VSV mRNA to mRNP Protein

irradiation[a]	cpm[b] of aqueous phase (%)	cpm[b] of organic plus interphase (%)
+	6480 (18)	29520 (82)
−	24654 (68)	11820 (32)

[a] CHO cells were infected for 3 h in the presence of [^{3}H]uridine (10 μCi/mL, 45 Ci/mmol) at which time polysomes were disrupted in vivo by addition of puromycin. Half the cells (1×10^8) were irradiated with UV light according to Wagenmakers et al. (1980). The [^{3}H]mRNP fraction from irradiated and unirradiated cells was purified as described under Materials and Methods. [b] Purified ^{3}H-labeled mRNP (35 000 cpm) was extracted with phenol (Rose & Knipe, 1975). The Cl_3CCOOH-precipitable radioactivity in both aqueous and organic phases was determined.

FIGURE 1: Protein synthesis during VSV infection. Growing CHO cells were collected and suspended in buffered medium at 2×10^6 cells/mL. The cells in the spinner flasks were incubated for 30 min at 34 °C and then infected at a multiplicity of 10. Actinomycin D (5 μg/mL) was added. At each time indicated in the figure, 2 mL was removed to a 13 × 100 mm glass test tube. ^{14}C amino acid mix (Amersham; specific activity 0.05 mCi/mL, 54 mCi/matom) was added, and the cells were stirred by means of a small magnetic stirring bar. After 1 h duplicate 1-mL samples were mixed with an equal volume of 10% Cl_3CCOOH, boiled for 10 min, cooled, and filtered through Whatman GF/C glass fiber filters which were counted in a Beckman LS-100 liquid scintillation spectrometer. Each point is an average of the two samples taken. The uninfected samples are indicated by crosshatching. The first samples were labeled for only 0.5 h. Therefore, the cpm were doubled. This experiment presents one of three performed, all of which gave similar results.

of radioactive amino acids incorporated into hot Cl_3CCOOH-insoluble material was determined. Incorporation at each interval is linear. Incorporation into protein in infected cells decreased to about 37% of the initial rate, whereas the rate in uninfected cells was essentially unchanged.

Inhibition of in Vitro Protein Synthesis by mRNP and Nucleocapsid. Since accumulation of mRNP and nucleocapsid occurs concomitantly with in vivo inhibition of host and viral protein synthesis (Rosen et al., 1982), we tested whether they could inhibit in vitro protein synthesis. All in vitro experiments were performed with both wheat germ and reticulocyte lysates. Only the reticulocyte data are presented, although the wheat germ system gave identical results. Small amounts of either mRNP or nucleocapsid were found to inhibit translation in the in vitro extract although mRNP was the better inhibitor (Figure 2). Their ability to block in vitro translation was not due to nonspecific addition of protein to the reticulocyte extract as bovine serum albumin had no effect (data not shown) and uninfected or early after infection cell extracts similarly processed had no effect. In addition, particles purified only by sucrose gradient centrifugation were also inhibitory.

Reversal of Particle-Mediated Inhibition of in Vitro Protein Synthesis. Recently, Centrella et al. (1982) showed that

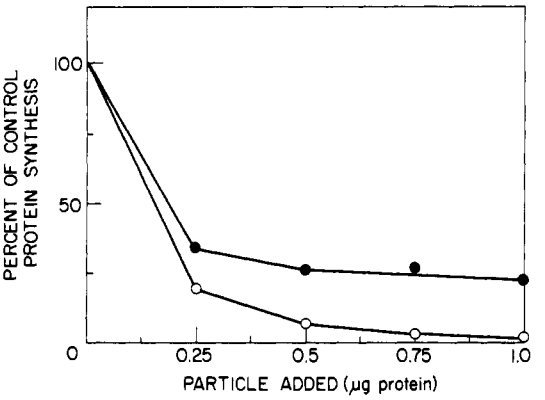

FIGURE 2: Inhibition of in vitro protein synthesis by mRNP and nucleocapsid. Nucleocapsids or mRNPs (1 μg with respect to protein) were added to reticulocyte lysates containing [^{35}S]methionine (25 μCi) in a total volume of 15 μL. Incubation was for 15 min at 30 °C, at which time 3 μg of phenol-purified 4.5 h VSV RNA in 2 μL was added to each reaction mixture. The reactions were incubated at 30 °C for an additional hour at which time 10 μL was removed and assayed for incorporation of [^{35}S]methionine into Cl_3CCOOH-precipitable protein. The incorporation in the control translation (no added mRNP or nucleocapsid) was 602 000 cpm above background. (●) Plus nucleocapsid; (○) plus mRNP.

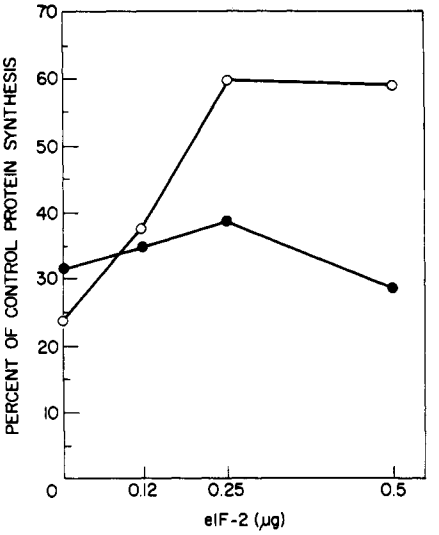

FIGURE 3: Reversal of mRNP and nucleocapsid induced inhibition of protein synthesis by purified rabbit reticulocyte eIF-2. See legend to Figure 2 for details of experiment. Control translation values (no added mRNP or nucleocapsid) varied between 246 000 cpm in the absence of added rabbit reticulocyte eIF-2 and 398 000 cpm in the presence of 0.5 μg of eIF-2. Homogeneous eIF-2 was prepared by the procedure of Benne et al. (1976). All values are given as percent of control protein synthesis with the indicated amount of eIF-2 and the absence of mRNP or nucleocapsid. (●) plus nucleocapsid; (○) plus mRNP.

cell-free extracts of VSV-infected L cells are deficient in functional eIF-2. We wondered whether the reason for this was because one or both of the particles bound the initiation factor, rendering it incapable of participating in protein synthesis. If this were so, then the addition of exogenous eIF-2 to reaction mixtures that were inhibited by excess particle would reverse the inhibition. We used eIF-2 purified to homogeneity in this study. Purified eIF-2 was indeed able to partially reverse mRNP-induced inhibition but not inhibition caused by nucleocapsid (Figure 3). Reversal of mRNP inhibition is presumably not complete because eIF-2 at higher concentrations is also inhibitory, as has been shown in other systems (Benne et al., 1980), or alternatively another factor interacts with EIF-2 and either does not permit complete

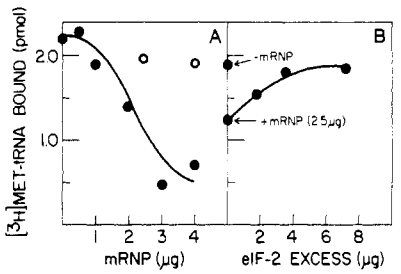

FIGURE 4: (A) Inhibition of ternary complex formation (left panel). The reaction was carried out as previously described (Siekierka et al., 1982). The reaction mixture contained 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 2 mM β-mercaptoethanol, 60 μM GTP, 3 pmol of [^{3}H]methionine-tRNA (50 000 cpm/pmol), and 3.6 μg of eIF-2. The indicated amounts of particle (determined as protein content) were added, and the reaction mixture was incubated for 5 min at 30 °C. The mixture was filtered through a Millipore nitrocellulose filter, washed with 10 mL of buffer containing 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM magnesium acetate, and 2 mM β-mercaptoethanol. The filters were transferred to glass scintillation vials, and 1 mL of methylcellulose (Fisher Scientific Co., Springfield, NJ) was added. The filters were dissolved with mechanical shaking and after adding 10 mL of Hydrofluor (National Diagnostics, Somerville, NJ) were counted in a Beckman LS100 scintillation spectrometer. (●) mRNP added; (○) nucleocapsid added. (B) Reversal of inhibition by addition of excess eIF-2 (right panel). Ternary complex formation was carried out as in (A). Homogeneous eIF-2 was added in excess of the amount normally present in the assay (3.6 μg). The mRNP (2.5 μg) was added last to the reaction mixture.

reversal of inhibition or is necessary for complete reversal.

Inhibition of Ternary Complex Formation by mRNP. eIF-2 is required for ternary complex formation, i.e., the formation of the eIF-2·GTP·Met-tRNA complex, the first step in initiation of protein synthesis (Dettman & Stanley, 1972; Schoemaker & Schimmel, 1974). For this reason, mRNP and nucleocapsid were tested for their ability to inhibit ternary complex formation. Indeed, complex formation was inhibited approximately 75% (Figure 4A) by concentrations of mRNP comparable to those that inhibited in vitro protein synthesis (compare with Figure 2). The inhibition could be reversed by addition of excess eIF-2 (Figure 4B). In contrast, nucleocapsid gave only about 10% inhibition.

Neither particle significantly inhibited poly(U)-directed synthesis of polyphenylalanine in reticulocyte lysates. In this experiment, the control incorporation of [^{3}H]phenylalanine into hot Cl_3CCOOH-insoluble material was 3190 cpm, whereas that in the presence of 2 μg/25 μL of reaction was 2427 and 3580 cpm for nucleocapsid and mRNP, respectively. The results indicate that mRNP does not inhibit the elongation step in protein syynthesis, as eIF-2 is not required for poly-(U)-directed protein synthesis.

Discussion

We recently characterized an mRNP that accumulates during infection of CHO cells by VSV (Rosen et al., 1982). It consists of all five VSV mRNAs and almost exclusively viral N protein. The particle is not nucleocapsid as it has a different buoyant density, macromolecular composition, and microscopic structure. The mRNP sequesters a large fraction of the VSV mRNA present during infection. We believe that the particle is involved in the translational control of VSV mRNA because the large amount of mRNA in the mRNP is apparently unavailable for translation.

Our present investigation points to a dual role for the mRNP in protein synthesis. We show that in addition to sequestering VSV mRNA, the particle inhibited in vitro protein synthesis when added to an in vitro reticulocyte protein synthesis lysate (Figure 2). The inhibition could be partically reversed by addition of homogeneous rabbit reticulocyte eIF-2 to the inhibited reticulocyte lysate (Figure 3). Presumably, mRNP-bound or -inactivated eIF-2 results in a deficiency of the initiation factor. The most compelling information that indicated that the mRNP specifically inhibited initiation by interfering with eIF-2 action was that the particle inhibited the formation of the eIF-2·GTP·Met-tRNA complex (ternary complex) (Figure 4A) but not polyphenylalanine synthesis. In addition, the addition of excess eIF-2 reversed the inhibition caused by mRNP (Figure 4B). Nucleocapsid had little effect on either of these reactions. Since poly(U)-directed protein synthesis does not require eIF-2, these results support the idea that the mRNP inhibits by preventing initiation and not by blocking the steps involved in peptide elongation. In support of the idea that mRNP interacts with eIF-2 is the finding that neither tRNA nor GTP reversed mRNP inhibition of in vitro protein synthesis in lysates (data not presented). In view of these findings, one might conclude that eIF-2 was limiting during infection because of binding or inactivation by mRNP, and this accounted for the inhibition of total protein synthesis observed late in infection. Our data do not address the question of how host protein synthesis is selectively inhibited during infection. The mechanisms by which a loss in eIF-2 activity might result in preferential translation of viral mRNA have already been discussed (Centrella & Lucas-Lenard, 1982).

The data we present are in agreement with those of Centrella & Lucas-Lenard (1982), who recently demonstrated that addition of partially purified mouse eIF-2 to extracts derived from VSV-infected mouse L cells (3.5 h after infection at 37 °C) effected almost complete recovery of the protein synthetic capacity of the extract. These investigators further found that salt wash preparations from ribosomes of infected and noninfected cells contained the same amount of eIF-2, although the initial rate of ternary complex formation by the factor from infected cell ribosomes apparently was somewhat slower than that from control cells. It is possible that the reason why Centrella & Lucas-Lenard (1982) were able to isolate equal amounts of eIF-2 from infected and noninfected cell ribosomes even though the extracts were ostensibly deficient in eIF-2 was because their extraction with high salt released the eIF-2 bound to the mRNP which cosediments with ribosomes in cell-free extracts (Rosen et al., 1982). The eIF-2 released from the mRNP would be active (or would be activated in the in vitro protein synthesis system) in carrying out ternary complex formation.

Recently, Thomas & Wagner (1983) also showed that initiation of translation was the site of VSV inhibition of protein synthesis in L cells. They found the factor(s) responsible was (were) located primarily in the 0–40% ammonium sulfate insoluble fraction of ribosome-associated proteins, which contains primarily eIF-3 and eIF-4B. They have, however, not shown directly which protein is responsible. Although the 0–40% fraction is enriched with respect to eIF-3 and eIF-4B, these authors do not state whether eIF-2 is also present. We have used eIF-2 purified to homogeneity for our studies, so we can be reasonably sure that the factor in question is not eIF-3 or eIF-4B. Moreover, the factor involved in VSV inhibition of protein synthesis in lysates of *Aedes albopictus* infected with VSV was found in the 40–70% ammonium sulfate fraction (Gillies & Stollar, 1982), in contrast to the results obtained by Thomas & Wagner (1983). It is possible that in addition to eIF-2, eIF-3 and/or eIF-4B (or another factor present in the 0–40% fraction) is also active or additive with eIF-2. This remains to be clarified.

It is noteworthy that nucleocapsid was also able to inhibit in vitro protein synthesis, although this inhibition was not reversed by added eIF-2. Consequently, it is possible that nucleocapsid also has a role in regulating protein synthesis but does so by a mechanism different from that achieved by the mRNP. Our future studies are directed to understanding more fully both mechanisms of protein synthesis regulation occurring during VSV infection.

Recently, Jones & Ehrenfeld (1983) isolated mRNP particles from HeLa cells doubly infected with poliovirus and VSV. The VSV mRNPs were used as a model for cell mRNPs. The mRNPs isolated by these authors appear to be different by a number of criteria from the ones we describe. Ours sediment in sucrose gradients at about 120 S and contain only viral N protein, and the viral mRNAs contained are not translatable when mRNPs are added to an in vitro protein synthesis system. The mRNPs described by Jones and Ehrenfeld sediment at about 40 S when dissociated from polysomes and contain a large number of host proteins, and the mRNP is translatable in vitro. It is possible that some proteins are stripped from the particle we describe during purification on CsCl density gradients. We do, however, know that the mRNP purified by sedimentation through sucrose gradients is not translatable in vitro and is as fully capable of inhibiting protein synthesis in reticulocyte lysates as the CsCl-purified particles. We, therefore, have to assume that the mRNPs isolated by Jones and Ehrenfeld are not the same as the ones we isolated.

References

Benne, R., Wong, C., Luedi, M., & Hershey, J. W. B. (1976) *J. Biol. Chem. 251*, 7675–7681.

Benne, R., Kasperaitis, M., Voorma, H. A., Ceglarz, E., & Legocki, A. B. (1980) *Eur. J. Biochem. 104*, 109–117.

Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–88.

Centrella, M., & Lucas-Lenard, J. (1982) *J. Virol. 41*, 781–791.

Dettman, G., & Stanley, W. (1972) *Biochim. Biophys. Acta 227*, 124–133.

Dunigan, D. D., & Lucas-Lenard, J. M. (1983) *J. Virol. 45*, 618–626.

Ehrenfeld, E., & Lund, H. (1977) *Virology 80*, 297–308.

Gillies, S., & Stollar, V. (1982) *Mol. Cell. Biol. 2*, 1174–1186.

Greenberg, J. R. (1979) *Nucleic Acids Res. 6*, 715–732.

Jaye, M. C., Godchaux, W., III, & Lucas-Lenard, J. (1982) *Virology 116*, 148–162.

Jones, C. L., & Ehrenfeld, E. (1983) *Virology 129*, 415–430.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lodish, H. F., & Porter, M. (1980) *J. Virol. 36*, 719–733.

Lodish, H. F., & Porter, M. (1981) *J. Virol. 38*, 504–517.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Lynch, K. R., Pennica, D., Ennis, H. L., & Cohen, P. S. (1981) *Virology 108*, 277–285.

McAllister, P. E., & Wagner, R. R. (1976) *J. Virol. 18*, 550–558.

Mudd, J. A., & Summers, D. F. (1970) *Virology 42*, 328–340.

Nuss, D. L., Oppermann, H., & Koch, G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1258–1262.

Pelham, H. R. B., & Jackson, R. J. (1976) *Eur. J. Biochem. 67*, 247–256.

Pennica, D., Lynch, K. R., Cohen, P. S., & Ennis, H. L. (1979) *Virology 94*, 484–487.

Roberts, B. E., & Patterson, B. M. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2330–2334.

Rose, J. K., & Knipe, D. (1975) *J. Virol. 15*, 994–1003.

Rosen, C. A., Ennis, H. L., & Cohen, P. S. (1982) *J. Virol. 44*, 932–938.

Schnitzlein, W. M., O'Banion, M. K., Poirot, M. K., & Reichmann, M. E. (1983) *J. Virol. 45*, 206–214.

Schoemaker, H. J., & Schimmel, P. R. (1974) *J. Mol. Biol. 84*, 503–513.

Siekierka, J., Mauser, L., & Ochoa, S. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2537–2540.

Stampfer, M., Baltimore, D., & Huang, A. S. (1969) *J. Virol. 4*, 154–161.

Stanners, C. P., Francoeur, A. M., & Lam, T. (1977) *Cell (Cambridge, Mass.) 11*, 273–281.

Thomas, J. R., & Wagner, R. R. (1982) *J. Virol. 44*, 189–198.

Thomas, J. R., & Wagner, R. R. (1983) *Biochemistry 22*, 1540–1546.

Valentine, R. C., Shapiro, B. M., & Stadtman, E. R. (1968) *Biochemistry 7*, 2143–2152.

Wagenmakers, A. J. M., Reinders, R. J., & Van Venrooij, W. J. (1980) *Eur. J. Biochem. 112*, 323–330.

Wagner, R. R. (1975) in *Comprehensive Virology* (Fraenkel-Conrat, H., & Wagner, R. R., eds.) Vol. 4, pp 1–95, Plenum Press, New York.

Wertz, G. W., & Youngner, J. S. (1972) *J. Virol. 9*, 85–89.