Proc. Nat. Acad. Sci. USA
Vol. 72, No. 7, pp. 2545–2549, July 1975
Biochemistry

Coupled *in vitro* transcription and translation of vesicular stomatitis virus messenger RNA

(vesicular stomatitis virus ribonucleoprotein cores/virion-associated RNA polymerase/cell-free protein synthesis/slab gel electrophoresis)

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Communicated by E. Peter Geiduschek, April 15, 1975

ABSTRACT **The virion transcriptase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) of vesicular stomatitis virus was fully active when ribonucleoprotein cores from purified virions were added to cell-free protein synthesizing systems of eukaryotic origin. Synthesis of mRNA was linear for at least 3 hr and the newly synthesized viral mRNA was efficiently utilized for the synthesis of viral proteins N (nucleoprotein), NS, and M (matrix); small amounts of a putative G (glycoprotein) protein precursor and several unidentified polypeptides were regularly synthesized. The ratio of the various newly synthesized viral proteins was identical after different periods of coupled mRNA and protein synthesis. Identical proteins were obtained when the cell-free protein synthesizing systems were programmed with purified VSV mRNA synthesized *in vitro*. No detectable L protein was synthesized, even though transcripts complementary to the complete viral genome were detectable in the mRNA preparation by hybridization.**

Vesicular stomatitis virus (VSV) is a rhabdovirus containing a single-stranded RNA genome with a molecular weight of 3.6 to 4 × 10^6 (1, 2). The five viral structural proteins (3–5) are the glycoprotein (G), the matrix protein (M), the nucleoprotein (N) and two minor proteins (L and NS). VSV also has a virion-associated RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) which can synthesize RNA complementary to the viral genome *in vitro* (6, 7). The *in vitro* RNA products have molecular weights ranging from 0.2 to 1 × 10^6 (8) and are polyadenylylated (9, 10).

Polysomes from VSV-infected cells contain two size classes of virus-specific mRNA (11, 12): 12–18S RNAs, which are similar in size to the *in vitro* transcriptase product, and 28S RNA. The latter class is not found in the *in vitro* product RNA (8, 13). Morrison *et al.* (14) have been able to translate polysomal mRNA isolated from VSV-infected cells into virus-like proteins in cell-free extracts of eukaryotic origin. The 12–18S RNA directed the synthesis of proteins co-migrating on polyacrylamide gels with the viral N, NS, and M proteins, and the 28S RNA coded for the L protein. No unequivocal evidence for the synthesis of the viral G protein was presented by these authors.

While this work was in progress it was shown by Both *et al.* (15) that the 12–18S RNA synthesized *in vitro* can function as mRNA. In cell-free extracts of wheat embryos, this RNA directed the synthesis of proteins similar to the viral N, NS, and M proteins and possibly a nonglycosylated G protein precursor. Both *et al.* (15) also separated the 12–18S RNA on sucrose gradients into fractions with different coding capacity, suggesting that the *in vitro* RNA consists of several monocistronic mRNAs each of which can code for an individual protein.

In this publication we report the translation of VSV *in vitro* mRNA in cell-free extracts from wheat embryos (confirming the results of Both *et al.*, ref. 15), Krebs II ascites cells, and rabbit reticulocytes. We also show that the transcription and translation processes can be coupled in one *in vitro* system by adding VSV ribonucleoprotein cores directly to cell-free protein synthesizing systems. The proteins synthesized under direction of purified VSV *in vitro* mRNA as well as in the coupled transcription–translation system are mainly the viral N, NS, and M proteins, and several unidentified bands, including small amounts of a possible precursor of the viral G protein. These proteins represent only about 50% of the genetic information of the virus. Although transcripts of the residual 50% can be detected by hybridization in the *in vitro* synthesized mRNA, no detectable amounts of the viral L protein are synthesized in the different cell-free systems.

MATERIALS AND METHODS

Cells and Virus. The Indiana serotype of VSV was grown in BHK 21 (baby hamster kidney) cells and purified by sucrose gradient centrifugation and subsequent potassium tartrate gradient centrifugation, as described elsewhere (16). The purified virus was stored at 0° in 10 mM Tris·HCl buffer, pH 7.6.

Preparation of Ribonucleoprotein Cores. Purified virus at a concentration of about 1 mg/ml was treated with 0.5% Nonidet P 40 in 50 mM Tris·HCl, pH 7.6, 5 mM Mg-acetate, 0.1% 2-mercaptoethanol, and 250 mM KCl at 0°, layered immediately on a discontinuous glycerol gradient [lower layer: 1.5 ml of 75% (v/v) glycerol, upper layer: 1.5 ml of 25% (v/v) glycerol, containing 10 mM Tris·HCl, pH 7.6], and centrifuged at 2° in the Spinco Ti 50 rotor at 40,000 rpm for 60 min. The ribonucleoprotein cores in the pellet were free of the virion G and M proteins, and recovery of transcriptase activity was usually 80–100% (not shown). The cores were resuspended in a minimal volume of 10 mM Tris·HCl, pH 7.6, and used immediately for *in vitro* synthesis of VSV mRNA or for coupled *in vitro* transcription and translation.

***In Vitro* Synthesis and Purification of VSV mRNA.** Ribonucleoprotein cores were incubated in 100 mM KCl, 50 mM Tris·HCl, pH 7.6, 5 mM Mg-acetate, 0.1% 2-mercap-

Abbreviations: VSV, vesicular stomatitis virus; viral structural proteins: G, glycoprotein; M, matrix protein; N, nucleoprotein; L and NS, minor proteins.

toethanol, and 1 mM each of the four ribonucleoside triphosphates at 28° for 3 hr. The reaction was terminated by the addition of EDTA (15 mM final concentration) and nuclease-free CsCl to a density of 1.36, and the mRNA was isolated by pelleting it at 40,000 rpm and 2° for 16–22 hr in the Spinco SW 50.1 rotor (J. Perrault, unpublished). VSV mRNA purified by this simple one-step procedure was free of detectable amounts of template RNA (J. Perrault, unpublished) and contained only minimal amounts of free ribonucleoside triphosphates. The RNA was resuspended in H_2O and used either directly or after reprecipitation with ethanol for cell-free protein synthesis.

Globin mRNA. Globin mRNA was extracted from rabbit reticulocyte polyribosomes with sodium dodecyl sulfate–deoxycholate as described by Stewart *et al.* (17) and purified by sucrose gradient centrifugation (18).

Cell-Free Protein Synthesis. The protein synthesizing systems used in these experiments were prepared from Krebs II ascites cells and rabbit reticulocytes as described before (18), and from wheat embryos by modifications of procedures described by others (19–21). The Krebs II ascites system was supplemented with initiation factors prepared from rabbit reticulocytes or rat liver by a modification of previously described methods (18, 22).

Standard reaction mixtures of 100 μl contained 10 μl of S-30 extract, 50 μg of initiation factors (ascites system only), mRNA or ribonucleoprotein cores as indicated, 1 mM each of the four ribonucleoside triphosphates, 15 mM creatine phosphate, 10 μg of creatine kinase, 80 mM KCl (ascites and reticulocyte systems) or 55 mM K-acetate (wheat embryo system), 5.5 mM Mg-acetate, 40 mM Tris·HCl, pH 7.6, and 0.1% 2-mercaptoethanol. To follow protein synthesis, we added either 5 μCi of [^{35}S]methionine (Amersham/Searle Corp., specific activity 150–300 Ci/mmol) or 5 μCi of [^{3}H]valine (Schwarz/Mann, specific activity 16 Ci/mmol); the concentrations of the appropriate unlabeled amino acids were 30 μM. Incubation was at 28°. At the indicated times aliquots were precipitated on Whatman 3MM filter paper strips in 10% trichloroacetic acid, washed, and analyzed in a liquid scintillation counter as described (18). When RNA synthesis was followed the radioactive amino acids were replaced by the appropriate unlabeled amino acids, the UTP concentration was 0.1 mM, and 0.5 μCi/100 μl of ^{3}H-labeled UTP (Schwarz/Mann) was added. Aliquots were spotted on Whatman DE 81 paper discs, washed, and analyzed for radioactivity according to the method of Blatti *et al.* (23).

Polyacrylamide Gel Electrophoresis and Autoradiography. Sodium dodecyl sulfate (final concentration 1%) and 2-mercaptoethanol (1%) were added to 10 μl aliquots of the protein synthesis assays. The samples were then heated for 2 min at 100°, and subjected to electrophoresis on analytical 10% polyacrylamide–dodecyl sulfate slab gels following the procedure of Laemmli (24). Electrophoresis was at 10 mA for 4 hr, and the gels were stained, destained, and dried *in vacuo*. Autoradiograms were obtained by exposure to Kodak RP Royal X-Omat medical x-ray film for the times indicated in the figure legends.

RNA·RNA Hybridization. Annealing reactions were carried out in sealed glass capillaries at 70° for 20 hr as described elsewhere (16) except that the phosphate buffer concentration was 0.48 M. After annealing, 10 μg/ml of pancreatic ribonuclease and 5 units/ml of T_1 ribonuclease were added for 30 min at 37°. Aliquots were then spotted on Whatman DE 81 paper discs, washed, and analyzed according to Blatti *et al.* (23).

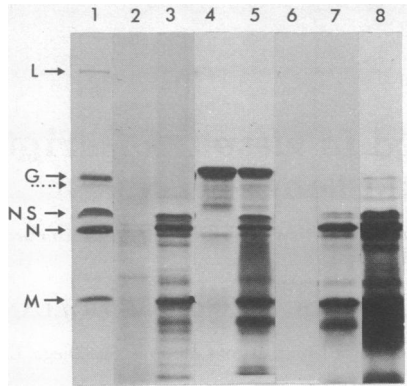

FIG. 1. Analysis of proteins synthesized in response to VSV *in vitro* mRNA in different protein synthesizing systems. VSV mRNA was transcribed *in vitro*, purified, and translated in cell-free protein synthesizing systems from Krebs II ascites cells, rabbit reticulocytes, and wheat embryos, as described in *Materials and Methods*. Aliquots were analyzed by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis (24) and autoradiography. The results of different experiments are summarized in this figure. The slots contain: [^{14}C]valine-labeled VSV proteins extracted from infected cells (*1*), proteins synthesized in the Krebs II ascites extract without (*2*) and with (*3*) addition of VSV *in vitro* mRNA, proteins synthesized in the rabbit reticulocyte extract without (*4*) and with (*5*) VSV *in vitro* mRNA, and proteins synthesized in the wheat embryo extract without (*6*) and with (*7*) VSV *in vitro* mRNA. The autoradiograms shown in slots 1–7 were exposed for 2 days. Slot 8 shows the same *in vitro* product as slot 7, but exposed for 10 days. The position of the putative G protein precursor is indicated by the dotted arrows. This band was not seen in the control (without mRNA) exposed for 10 days. Globin, the main endogenous product of the reticulocyte system, runs off the gels under these conditions of electrophoresis.

RESULTS

Translation of purified VSV *in vitro* mRNA in cell-free extracts of different eukaryotic cells

VSV mRNA was synthesized *in vitro* by virus ribonucleoprotein cores and purified as described in *Materials and Methods*. Cores prepared from 5 mg of purified virus contained about 50 μg of template RNA and yielded approximately 150 μg of mRNA in 3 hr. This mRNA was translated in cell-free extracts prepared from Krebs II ascites cells, wheat embryos, and rabbit reticulocytes, and the resulting proteins were analyzed by dodecyl sulfate–polyacrylamide slab gel electrophoresis. Fig. 1 shows that VSV mRNA synthesized *in vitro* principally directed, in all three cell-free systems, the synthesis of three of the five viral proteins (the NS, N, and M proteins), and a reproducible pattern of proteins not corresponding in size to any of the major viral proteins. The relative proportions of the different proteins synthesized *in vitro* were similar in the three cell-free extracts, except that the NS protein was synthesized less efficiently in the wheat embryo extract (Fig. 1, compare slots 3, 5, and 7). The NS protein did not co-migrate exactly with the authentic viral protein, but it has been further identified as NS by using polyacrylamide gels containing sodium phosphate buffer instead of Tris–glycine. Under these conditions the NS protein characteristically changes its position relative to the other viral proteins and migrates faster than the N protein (25). An extended exposure time of the autoradiograms showed that the Krebs II ascites and wheat embryo systems synthesized small quantities of a protein also present in infected cell extracts labeled with radioactive amino acids (Fig. 1, slots 1 and 8). This protein has tentatively been iden-

Table 1. Hybridization of [^{3}H]UTP-labeled VSV virion RNA and VSV mRNA synthesized *in vitro*

	RNA hybridized	
	cpm	% ribonuclease resistance
No treatment	2285	—
Ribonuclease resistance*	148	6.5
Self-annealing†	202	8.8
4 μg of mRNA	2294	100.4
1.2 μg of mRNA	2368	103.6
0.4 μg of mRNA	2332	102.1
0.12 μg of mRNA	1517	66.4
0.04 μg of mRNA	797	34.9

^{3}H-labeled virion RNA obtained from purified VSV labeled during replication with [^{3}H]uridine was provided by Dr. J. Perrault. Unlabeled VSV *in vitro* mRNA was added in the amounts indicated and annealed and digested with RNAse as described in *Materials and Methods*.

* No mRNA added, sample was kept at 0° prior to ribonuclease digestion.

† No mRNA added.

tified as the nonglycosylated precursor of the viral G protein (15). It has very recently been found by Both *et al.* (26) that a protein with similar electrophoretic mobility is coded for by mRNA extracted from membrane-bound polyribosomes of VSV-infected cells. This protein has a similar tryptic peptide pattern as the viral G protein, justifying the assumption that it is indeed the G protein precursor. The putative G protein precursor was not detected in the reticulocyte *in vitro* product because endogenous proteins migrate at the same position in the polyacrylamide gel (Fig. 1, slots 4 and 5). These results generally confirm the findings of Both *et al.* (15). The viral L protein was not synthesized in detectable quantities, although hybridization studies (Table 1) showed that the transcription of the viral genome was complete under the conditions employed here, i.e., virtually 100% of the template RNA was protected from RNAse digestion by product RNA. This is in agreement with results of others (refs. 7 and 16, J. Perrault, unpublished).

Coupled *in vitro* transcription–translation system

VSV ribonucleoprotein cores in the presence of cell-free protein synthesizing extracts of Krebs II ascites cells or wheat embryos were active in RNA synthesis by the virion-associated RNA polymerase for at least 3 hr (Fig. 2a). The newly synthesized RNA was used as mRNA by these extracts and translated into trichloroacetic-acid-precipitable proteins (Fig. 2b). We usually observed a slight stimulation of transcriptase activity by the cell-free extracts, possibly due to a stabilization of the ribonucleoprotein cores. Sucrose gradient analysis of the product RNA synthesized in the presence of the wheat embryo extract revealed that it was similar in size (10–18 S) to that synthesized by VSV cores alone or detergent activated virus; however, in the presence of the Krebs II ascites extract almost all of the synthesized mRNA was found to be of smaller size (about 5 S, data not shown). No appreciable quantities of 28S mRNA were synthesized in either system.

Under optimal conditions the stimulation of protein synthesis in the Krebs II ascites and wheat embryo extracts in response to transcribing VSV ribonucleoprotein cores was

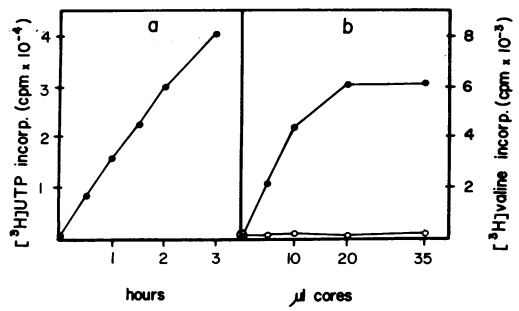

FIG. 2. (a) Kinetics of RNA synthesis by VSV ribonucleoprotein cores in the presence of a cell-free wheat embryo extract. VSV ribonucleoprotein cores were prepared from 3 mg of purified virus as described in *Materials and Methods* and resuspended in 200 μl of 10 mM Tris. Twenty-five microliters were added to a cell-free wheat embryo extract (total volume 50 μl) and incubated in the presence of [^{3}H]UTP at 28°. Aliquots (5 μl) were analyzed at the indicated times for [^{3}H]UTP incorporation as described in *Materials and Methods*.

(b) Stimulation of protein synthesis in a cell-free extract of wheat embryos by VSV ribonucleoprotein cores. Cores were prepared from 3 mg of virus as described in *Materials and Methods* and resuspended in 200 μl of 10 mM Tris-HCl, pH 7.6. The indicated amounts were added to a cell-free wheat embryo extract containing [^{3}H]valine as radioactive precursor, and incubated at 28° for 2 hr. The total volume was 100 μl. Aliquots (10 μl) were precipitated with trichloroacetic acid, washed, and analyzed for radioactivity (18). Complete system (●——●); without UTP and CTP (○——○). Under identical conditions saturating amounts of rabbit globin mRNA stimulated the incorporation of 14,674 cpm.

about 50% of that obtainable with rabbit globin mRNA. Protein synthesis in these coupled systems was dependent on mRNA transcription, as no stimulation occurred when two of the four ribonucleoside triphosphates (UTP and CTP) were omitted from the reaction mixture (Fig. 2b). Similarly, purified VSV virion RNA did not stimulate amino-acid incorporation (not shown).

The kinetics of *in vitro* protein synthesis in response to transcribing VSV ribonucleoprotein cores showed striking differences when compared with the translation of either globin mRNA or purified VSV *in vitro* mRNA (Fig. 3). The rate of protein synthesis in response to added mRNA was usually linear for 30–45 min and then gradually declined. In contrast, under coupled conditions there was an initial lag phase of 15–30 min, followed by linear rates of protein synthesis for up to 2.5 hr. These results were repeatedly observed in the wheat embryo extract (Fig. 3b). Similar kinetics were observed in the Krebs II ascites extract, although in this system the initial lag phase was less pronounced and the rate of protein synthesis declined after 1–1.5 hr (Fig. 3a). The rabbit reticulocyte extract was not used for the coupled *in vitro* transcription–translation reaction.

An analysis by gel electrophoresis of the proteins synthesized *in vitro* in the coupled transcription–translation systems revealed (Fig. 4) that the transcribing ribonucleoprotein cores directed the synthesis of the same proteins in similar relative proportions as the purified VSV *in vitro* mRNA (compare Fig. 1), namely the viral NS, N, and M proteins, small amounts of the putative G protein precursor (Fig. 4, slot 7), and a very similar pattern of proteins not corresponding in size to the major viral proteins. Moreover, the ratio of the different newly synthesized viral proteins was identical after short incubation times (30 min, Fig. 4, slot 5) and longer incubation times (60–150 min, Fig. 4, slot 6), showing that the transcription of at least three, and possibly four, of the

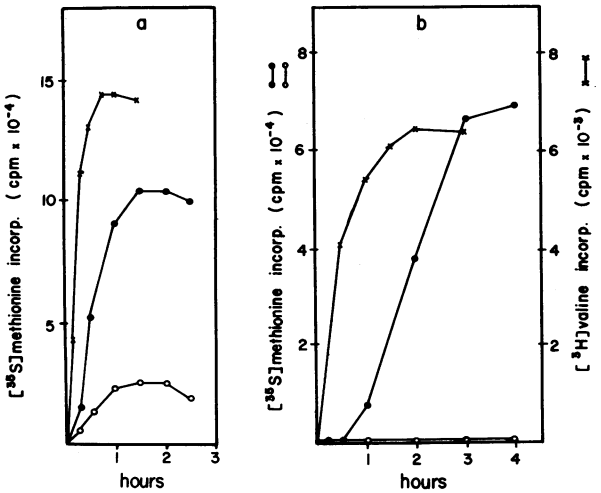

FIG. 3. (a) Kinetics of cell-free protein synthesis in a Krebs II ascites extract, without added mRNA (O——O), with 2 μg of VSV *in vitro* mRNA (×——×), or with 40 μl of VSV ribonucleoprotein cores containing about 8 μg of VSV template RNA (●——●) in a total volume of 100 μl. VSV *in vitro* mRNA and ribonucleoprotein cores were prepared as described in *Materials and Methods*. At the indicated times 5 μl aliquots were analyzed for [^{35}S]methionine incorporation into hot trichloroacetic-acid-precipitable proteins. The high endogenous protein synthesis in this experiment was due to a contamination of the rabbit reticulocyte initiation factors with mRNP. In similar experiments with rat liver initiation factors no endogenous protein synthesis was observed (not shown).

(b) Kinetics of cell-free protein synthesis in a wheat embryo extract, without added mRNA (O——O), with 4 μg of rabbit globin mRNA (×——×), or with 40 μl of VSV ribonucleoprotein cores (●——●) in a total volume of 100 μl. For details see above. Globin mRNA translation was done in an independent experiment with [^{3}H]valine rather than [^{35}S]methionine as radioactive precursor and included in this figure for the purpose of comparison. Background subtractions (zero time samples) were 1.1×10^4 ^{35}S cpm and 60 ^{3}H cpm, respectively. The high [^{35}S]methionine background was due to binding of acid-soluble methionine to the filter paper. It can be substantially reduced by addition of reducing agents to the 10% trichloroacetic acid wash.

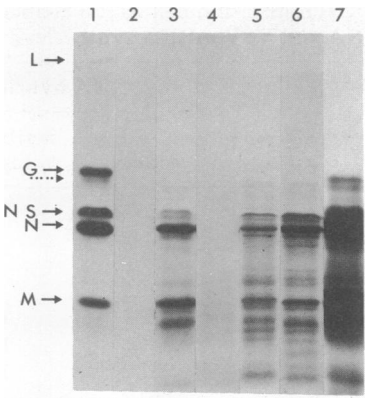

FIG. 4. Analysis of proteins synthesized in the coupled transcription–translation reaction. VSV ribonucleoprotein cores were prepared and added to cell-free protein synthesizing systems prepared from wheat embryos and Krebs II ascites cells as described in *Materials and Methods*. Aliquots (10 μl) were analyzed by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis (24) and autoradiography. The results of different experiments are summarized in this figure. The slots contain [^{14}C]valine-labeled VSV proteins extracted from infected cells (*1*), proteins synthesized in the wheat embryo system without (*2*) and with (*3*) addition of VSV ribonucleoprotein cores, proteins synthesized in the Krebs II ascites system without (*4*) and with addition of VSV ribonucleoprotein cores after 30 min (*5*) and 150 min (*6*) of coupled mRNA and protein synthesis. The autoradiograms shown in slots 1–6 were exposed for 2 days. Slot 7 shows the product after 3 hr of coupled *in vitro* mRNA and protein synthesis in the wheat embryo system (compare Fig. 3b). This autoradiogram was exposed for 10 days to show the presence of the putative G protein precursor (dotted arrows) and the absence of detectable amounts of the L protein.

five viral mRNAs initiates early in the reaction. Even after very long periods of linear mRNA and protein synthesis in the wheat embryo extract (compare Figs. 2 and 3) no detectable L protein was synthesized (Fig. 4, slot 7).

DISCUSSION

The results presented in this paper show that VSV ribonucleoprotein cores when added directly to protein synthesizing systems of eukaryotic origin are an excellent generating source of functional viral mRNA which is effectively translated into proteins (Figs. 2 and 3). It has been shown (7) that under *in vitro* conditions the transcription of the entire VSV genome is complete after 50–60 min. In our experiments mRNA synthesis was linear for at least 3 hr (Fig. 2a). This apparently complete transcription of the virus genome has been verified by hybridization studies (Table 1) showing that the entire VSV genome was transcribed under the conditions employed in these experiments. Since protein synthesis in the coupled systems was linear for at least 1 hr and as long as 2.5 hr (Fig. 3) conditions seemed adequate for complete transcription and translation of the entire genetic information of the virus. Nevertheless, the translation product consisted mainly of three of the five viral proteins (the NS, N, and M proteins) and possibly small amounts of a fourth viral protein, the unglycosylated precursor of the viral G protein (Fig. 4). The same result was obtained when VSV mRNA was transcribed *in vitro* for three hr and translated after purification (Fig. 1). The G, NS, N, and M proteins together represent about 50% of the genetic information of the virus, the rest being required to code for the L protein (molecular weight 1.5 to 1.9×10^5, refs. 5 and 25). Since transcripts representing 100% of the viral genome were present in the *in vitro* mRNA (Table 1), the absence of the L protein in the *in vitro* protein product (Figs. 1 and 4) indicates that the L protein mRNA was transcribed but not translated in the cell-free systems. This is most likely due to a degradation of the L protein mRNA, since no detectable quantities of full-size 28S mRNA were present in the *in vitro* transcription product (compare above). It cannot be ruled out, however, that small amounts of full-size 28S mRNA were synthesized in the *in vitro* systems, but not translated as efficiently as the other viral mRNA species, thus reflecting a possible translational control of VSV-specific protein synthesis. Consistent with this possibility is the fact that the viral L protein is present in infected cells in a much lower molar concentration than the other viral proteins (refs. 4 and 27, and compare the marker proteins in Figs. 1 and 4, slots 1), although all viral mRNAs seem to be present in equimolar amounts (28). The underrepresentation of the viral G protein precursor in the translation products might also be due to mRNA degradation. Another possibility is that specific conditions are necessary for the translation of the G protein mRNA which are not met by the *in vitro* systems. This latter possibility is supported by the findings that mRNA (14) or total cytoplasmic extracts (29) from VSV-infected cells also direct the synthesis of very little, if any, G protein *in vitro*

under conditions suitable for the VSV L, NS, N, and M protein mRNA translation.

The three different cell-free systems reproducibly synthesized a nearly identical pattern of discrete proteins not corresponding in size to the major viral proteins (Fig. 1, slots 3, 5, and 7). These proteins were also produced in the coupled transcription–translation reaction (Fig. 4, slots 3 and 6), and might be the products of premature termination during the transcription and/or the translation process. An intriguing possibility is that these polypeptides are viral proteins which are modified or prematurely terminated at defined signals on the mRNA molecules, having a function in the virus life cycle. This possibility is supported by the observation that polypeptides with the same electrophoretic mobility are present in extracts of VSV-infected cells, although in lesser amounts than are produced *in vitro* (M. Breindl and J. J. Holland, unpublished).

An interesting result is the finding that the protein products after short (30 min, i.e., shortly after the end of the lag period, Fig. 3) and long (150 min) periods of coupled transcription and translation in the Krebs II ascites extract are identical (Fig. 4, slots 5 and 6). Similar results were obtained with the wheat embryo extract (not shown). This shows that the transcription of at least three, and possibly four, of the five viral mRNAs initiates early in the reaction. However, more knowledge about the nature of the initial lag period (see below) as well as other parameters of the reaction is necessary to definitely decide whether there is synchronous or sequential transcription of the individual viral mRNA molecules.

A major characteristic of the coupled transcription–translation reaction is the 15- to 30-min lag phase in protein synthesis (Fig. 3), which is not seen for mRNA transcription (Fig. 2a). The cause for this lag period is not known. However, it can be calculated from the incorporation of radioactive ribonucleoside triphosphates of known specific activity into the *in vitro* product RNA that the VSV polymerase achieves 1- to 2-fold net RNA synthesis in 1 hr (compare Fig. 2a). Since most of the particles of a virus preparation are active in RNA transcription (13), 30–60 min are required to synthesize one genome equivalent of the virus (7), and it seems possible that the major portion of the lag period reflects the time necessary to complete and release the individual mRNA molecules. The coupled *in vitro* transcription–translation system characterized in this publication provides a most useful tool to study this and other problems of VSV-specific protein synthesis.

We thank Estelle Bussey for excellent technical assistance and Dr. J. Perrault for the gift of [^{3}H]uridine-labeled VSV virion RNA and for helpful discussion. This work was supported by Grant no. I0802 from the National Cancer Institute and by a research fellowship from the Deutsche Forschungsgemeinschaft.

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