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Transcribing Complexes in Cells Infected by Vesicular Stomatitis Virus and Rabies Virus

LOUIS PEREZ VILLARREAL AND JOHN J. HOLLAND
Department of Biology, University of California at San Diego, La Jolla, California 92037

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We analyzed cell extracts from BHK_{21} cells infected with vesicular stomatitis virus (VSV) and rabies virus for in vitro RNA polymerase activity. Cells infected with VSV B virions exhibited several complexes with in vitro RNA polymerase activity in sucrose gradients. These complexes synthesize VSV transcriptase product (4 to 18*S*) polyadenylated in RNA complementary to virion RNA. Cells infected with a high multiplicity of B virions and T particles show only one RNA polymerase complex active in vitro. This complex sediments at 110*S* and makes only small (2*S*) RNA. Carrier BHK_{21} cells persistently noncytopathically infected with VSV contain several complexes active in RNA polymerase, but both exhibit very low activity. Cytoplasms of cells noncytopathically infected with rabies virus also show very low levels of a complex containing RNA polymerase activity. No transcriptase nor any other in vitro polymerase activity could be found associated with purified rabies virions, although they do carry out primary transcription in cells treated with actinomycin D and cycloheximide before infection.

Vesicular stomatitis virus (VSV) and rabies virus are both bullet-shaped, enveloped viruses of the rhabdovirus group (11). Rhabdoviruses which have been reported to contain a virion-associated transcriptase include VSV (2), Kern Canyon virus (1), and lettuce necrotic yellow virus (15), but surprisingly, rabies virus has been reported to lack RNA polymerase activity under conditions in which VSV incorporates nucleoside triphosphates (17). A possible reason for this could be that the conditions necessary for rabies transcriptase differ from those of VSV. Alternatively, some host functions might be involved in transcription, or rabies may not have a virion transcriptase (this latter possibility seems unlikely).

This report confirms the observation of Sokol and Clark (17) that purified rabies virions lack a transcriptase which is detectable in vitro under assay conditions optimal for VSV virion transcriptase. Nevertheless, purified rabies virions do carry out primary transcription in infected cells pretreated with actinomycin D and cycloheximide. We also describe a comparative study of sedimentable complexes active in RNA polymerase isolated from the cytoplasm of normal and VSV-infected BHK_{21} cells. Our findings confirm the results of Galet et al. (8, 9) regarding polymerase activity associated with ribonucleoprotein complexes from VSV-infected cells, and extend them to rabies-virus-infected cells and to carrier cells persistently and noncytopathically infected with VSV.

MATERIALS AND METHODS

Wild-type VSV Indiana serotype was used for B virion and T particle infections except as described for the carrier culture. VSV was grown in monolayers of BHK_{21} cells, and B virions and T particles were purified as described (6). Rabies virus (HEP Flury strain), kindly supplied by H. Koprowski of the Wistar Institute, was propagated essentially as described by the Wistar group (18). BHK_{21} cells were grown in minimal essential medium (MEM) of Eagle plus 7% calf serum, but were infected at 33 C in MEM containing 0.4% bovine serum albumin (BSA) in place of serum. Plaque assays were carried out in MEM containing 0.4% BSA with agarose-suspended BHK_{21} cells as described by Sokol et al. Plaques were developed and counted after 7 days of incubation at 33 C. The identity of our infectious virus as rabies was conformed by neutralization with antirabies antiserum (also kindly provided by H. Koprowski of the Wistar Institute). The rabies virus pool employed had a titer of 7×10^8 PFU per ml and was stored frozen in small portions at −70 C until used to infect cells at an input multiplicity of approximately 30. The procedure employed for purifying rabies virions was identical to that described for VSV (6).

Isolation and assay of active polymerase complexes from infected cells. A 0.3-ml amount of cytoplasmic extract from normal or infected cells was layered on a 5-ml linear 5 to 20% sucrose gradient containing 0.066 M NaCl, 0.044 M KCl, 55 mM Tris-hydrochloride, pH 6.6. The gradients had a

0.2-ml cushion of 40% sucrose at the bottom. The gradients were spun in a Beckman SW50 rotor at 49,000 rpm at 4 C for either 30 min in the case of VSV cell extracts, or for 25 min in the case of rabies cell extracts. Gradients were fractionated into 20-drop fractions and collected in sterile tubes. Each fraction was tested for the presence of RNA polymerase complexes in the following way: 0.04 ml of each gradient fraction was mixed with 0.02 ml of a 3X transcriptase cocktail to give a final concentration of 0.06 M NaCl, 0.04 M KCl, 50 mM Tris-hydrochloride, pH 7.6, 0.1% mercaptoethanol, 1 mM each GTP, CTP, ATP (Calbiochem), 0.05 mM unlabeled UTP, 50 μCi of [^{3}H]UTP per ml (14 μCi/mmol from Schwartz). Samples were incubated at 28 C and 0.05-ml samples were spotted on Whatman DEAE-cellulose, washed, and counted at intervals as described (4).

Carrier culture. A carrier culture of BHK_{21} cells persistently infected with VSV was established as described elsewhere by J. J. Holland and L. P. Villarreal (Proc. Nat. Acad. Sci., in press). Briefly, the BHK_{21} cells were persistently infected with a mixture of B virions and short T particles of the ts 31 mutant of Prinagle, and surviving cells were cultivated. They continued to grow and produce virus despite periodic cytopathic degeneration of a majority of the cells (as described earlier for an L cell carrier culture by Wagner et al. [22]). After 3 months the carrier culture stabilized and has replicated like normal BHK_{21} cells since then without periodic crises of cytopathology. This persistent noncytocidal carrier state involves continuous production of small numbers of B virions and newly generated long T particles by all cells in the culture. These new long T particles are essential determinants of the persistent noncytocidal state since cloned B virions from carrier cells kill all BHK_{21} cells when infecting alone, but they readily establish new persistent noncytocidal carrier infections in the presence of long T particles from the carrier cells. Furthermore, the long T particles which arose in carrier culture are able to reestablish new carrier cultures when coinfecting with any B virion (with even the virulent wild-type B virion; J. J. Holland and L. P. Villarreal, Proc. Nat. Acad. Sci., in press).

RESULTS

RNA polymerase complexes in normal cells. In initial control experiments a cytoplasmic extract of uninfected BHK_{21} cells was sedimented in a sucrose gradient and assayed for RNA polymerase activity as described in Materials and Methods. As shown in Fig. 1, uninfected cell extracts contain an RNA polymerase activity sedimenting in two peaks, one at 80*S* and the other at 40*S*. The polymerase activity is a poly(U) polymerase that preferentially incorporates UTP (Table 1). This activity has been previously described by Burdon and Smellie (5) and later by Wykes and Smellie (26). In agreement with their results we find

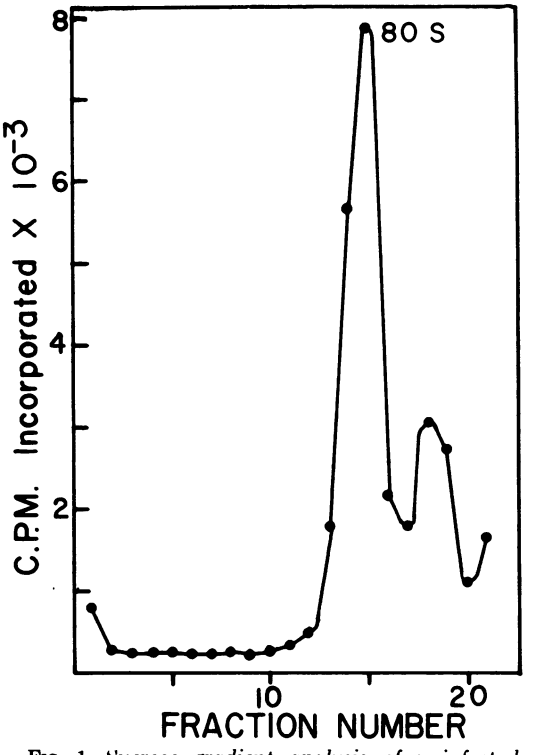

FIG. 1. *Sucrose gradient analysis of uninfected BHK_{21} cell cytoplasmic extract for RNA polymerase. BHK_{21} cells grown as a monolayer in 32-oz bottles were gently scraped off the glass into 0.15 M NaCl solution and pelleted in sterile test tubes by centrifuging for 5 min at 1,000 rpm in an International model PR-2 (small rotor). The supernatant was discarded and the cells were resuspended in an equal volume of sterile distilled water with 0.1% β-mercaptoethanol. After 10 min of swelling at 0 C the cells were homogenized in a Dounce homogenizer with 20 strokes. The nuclei and cellular debris were removed by centrifuging the samples in a sterile tube for 10 min at 2,000 rpm and carefully removing the supernatant with a Pasteur pipette. This supernatant served as the crude cytoplasmic extract. A 0.3-ml amount of cytoplasmic extract was layered on a 5 to 20% sucrose gradient and centrifuged for 30 min at 49,000 rpm in an SW50 rotor (as described in Materials and Methods). The gradients were fractionated and assayed for RNA polymerase activity at 28 C for 2 h as described in Materials and Methods.*

that the poly(U) polymerase does not require the other nucleoside triphosphates and preferentially utilizes UTP as a substrate in the presence of the other triphosphates (24-26). Table 1 demonstrates that the polymerase activity is almost undetectable prior to fractionating cytoplasm on a gradient. Over 90% of the activity was lost if the cytoplasmic extract was treated with 0.5% deoxycholate before loading on the gradient. Polymerase activity was greater at

28 C than at 36 C. When the cells were lysed in the presence of 5 mM $MgCl_2$ (Fig. 2A) all poly(U) polymerase activity cosedimented with the 80S monosome. If the cells were lysed in the presence of 6 mM EDTA, incubated for 1 h at 0 C and then briefly sonically treated (Fig. 2B), the remaining poly(U) polymerase activity still cosedimented with the 80S monosome. However, if the EDTA-lysed cells were fractionated immediately on the gradient and were not sonicated or incubated at 0 C, poly(U) polymerase activity was found associated with the 60 and 40S subunits as well as with 80S ribosome monomers (Fig. 2C). This suggests that the poly(U) polymerase is more tightly bound to ribosomes than to ribosomal subunits.

Sedimentable RNA polymerase complexes from cells infected with VSV B virions. We next analyzed the cytoplasm from cells infected for various times with cloned B virions of VSV at high multiplicity of infection (MOI) (under these conditions maximum yield of infectious virus is obtained with our strain of VSV Indiana and our strain of BHK_{21} cells between 9 and 12 h postinfection). Figure 3 confirms the findings of Galet et al. (9) in demonstrating that cytoplasmic extracts from cells infected with VSV contain (in addition to the 80S poly [U] polymerase) several virus-induced peaks of polymerase activity. The sedimentation rate and the relative activity of these polymerase complexes changes with time after infection and shows some variability in different experiments. At 6 h postinfection (Fig. 3A) a complex sedimenting at about 100 to 110S was most active in RNA polymerase. In some experiments complexes were also observed at about 140S and at about 170 and 200S (see Fig. 8A). The cellular poly(U) polymerase appears as a partially resolved shoulder or relatively small peak in each infected cell extract. At 11 h postinfection (Fig. 3B) most of the polymerase activity was present in the faster moving structures which sediment between 170 and 200S. The product RNA synthesized by all of these virus-induced structures sediments between 4 and 18S (Fig. 4). This is the size of the most abundant VSV mRNA species (2, 9, 10, 12, 14). Furthermore, the product RNA of our polymerase complexes contains up to 35% poly(A) (3, 8, 21) and anneals to B virions. This confirms the observations of Galet and Prevec (8) regarding slower-sedimenting complexes and extends them to show the presence and activity of faster sedimenting polymerase structures. These results also confirm that there is no detectable virion-sized RNA synthesized by either of these structures (in agreement with the report of Galet et al. [9]).

TABLE 1. *Characterization of poly(U) polymerase*[a]

^{3}H-labeled nucleotide	Nucleotide incorporated (μmol)
UTP	5.5
ATP	0.53
GTP	0.10
CTP	0.50
Complete system	**UTP incorporated (μmol)**
+APT, GTP, CTP	4.0
+GTP, CTP	8.3
+ATP, CTP	4.4
+ATP, GTP	7.9
At 28 C	6.9
At 37 C	2.4
+50 μg of actinomycin D per ml	6.5
Crude cytoplasmic extract	0.055
Total incorporation across gradient	36.0

[a] Active poly(U) polymerase was taken from the 80S region of a sucrose gradient similar to that of Fig. 1. Standard conditions for poly(U) polymerase assay were 50 μCi of [^{3}H]UTP per ml, 14.0 Ci/mmol, 0.06 M NaCl, 0.04 M KCl, 0.1% β-mercaptoethanol, 50 mM Tris-hydrochloride, pH 7.6. A 50-μliter amount of polymerase from a sucrose gradient was mixed with 50 μliters of 2X polymerase cocktail and incubated for 90 min at 28 C. A 50-μliter amount was then spotted on Whatman DEAE-cellulose and washed as described (4). Where indicated, the following deviations from standard conditions were employed: labeled nucleotides were at 50 μCi/ml with specific activities of 12.6 Ci/mmol ATP, 19.4 Ci/mmol GTP, 7 Ci/mmol CTP. Unlabeled nucleosidetriphosphates (Calbiochem) were at 1 mM.

RNA polymerase complexes from cells infected with VSV B virions plus T particles. Cells infected with infectious B virions at high MOI plus T particles at low MOI (approximately 3) show only a slightly altered pattern of transcribing structures in the cytoplasm (Fig. 5A), but when cells were infected with a combination of a high MOI of B virions and a high MOI of T particles (which yields mainly T particles) a markedly different pattern of polymerase activity was obtained (Fig. 5B). Only one polymerase complex (sedimenting at approximately 100S) was obtained. The product RNA synthesized by this complex sedimented at about 2S (Fig. 4C). Synthesis of 2S product RNA molecules is also characteristic of the in vitro polymerase activity associated with purified VSV T particles (16). Note that cellular

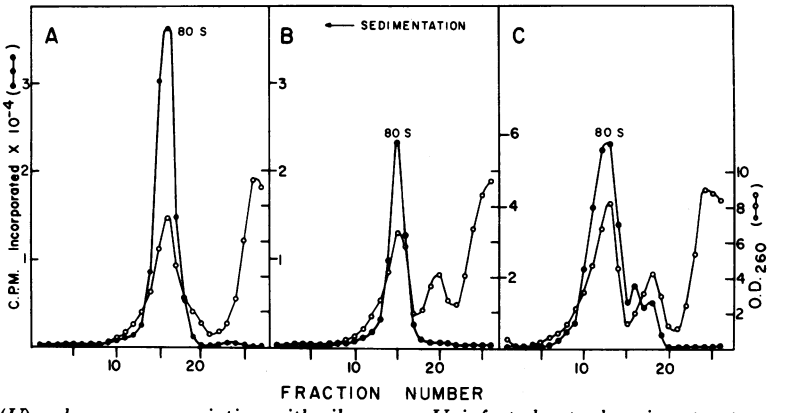

FIG. 2. *Poly(U) polymerase association with ribosomes. Uninfected cytoplasmic extracts were prepared as described in Fig. 1 with modifications as noted. (A) Cells were lysed in the presence of 5 mM $MgCl_2$ at 0 C. (B) Cells were lysed in the presence of 5 mM EDTA, and cytoplasmic extract was prepared, incubated at 0 C for 2 h, and sonically treated with a Branson sonifier model LS-75 at setting 2 for 10 s. (C) Cells were lysed in the presence of 5 mM EDTA and the cytoplasmic extracts were prepared with no incubation at 0 C or sonic treatment. A 0.3-ml amount of cytoplasmic extract was layered on a linear 5 to 20% sucrose gradient in 0.06 M NaCl, 0.04 M KCl, 50 mM Tris, and spun in a Spinco SW 50L rotor at 49,000 rpm at 4 C for 50 min in the case of panel A and B and for 75 min in the case of panel C. Gradients were fractionated into 20-drop fractions. Poly(U) polymerase assay was performed by mixing a 60-μliter portion of each gradient fraction with 30 μliters of a 3X polymerase cocktail to give a final concentration of salts and substrates as described in Table 1. The reactions were allowed to proceed for 90 min at 28 C and then 50 μliters was spotted on DEAE-cellulose, washed, and counted. Symbols: ●, counts per minute; O, optical density at 260 nm.*

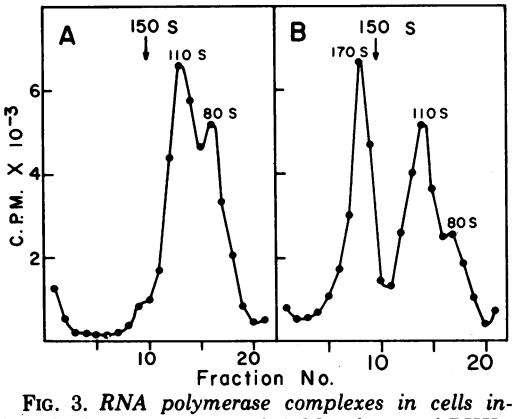

FIG. 3. *RNA polymerase complexes in cells infected with B virions of VSV. Monolayers of BHK_{21} cells were infected at an MOI of 200 with VSV. (A) Cell extracts prepared at 6 h postinfection. (B) Cell extracts prepared at 11 h postinfection. Extracts were analyzed on a sucrose gradient for RNA polymerase activity as described in Materials and Methods. Polymerase reaction was terminated at 90 min.*

poly(U) polymerase activity is greatly suppressed in T particle-infected cells, and that there is some "soluble" polymerase activity at the top of the gradient in Fig. 5.

Polymerase complexes from rabies virus-infected BHK_{21} cells. Rabies virus though morphologically very similar to VSV grows very slowly and is generally noncytocidal (17). To obtain transcribing complexes from rabies infected cells it was therefore necessary to allow infection to proceed much longer. At 48 h postinfection (Fig. 6B), a polymerase complex sedimenting at about 260*S* was barely detectable, but it was better resolved by 72 h postinfection (Fig. 6C). By 84 h postinfection the synthetically active complex at 260*S* was relatively quite prominent (Fig. 6D) (due to a sharp decline in the cellular poly[U] polymerase activity). Control cytoplasm from mock-infected BHK_{21} cells (Fig. 6A) retained most of its poly(U) polymerase activity and exhibited no detectable activity at 260*S*.

We have determined that the polymerase activity induced by rabies virus requires all four nucleoside triphosphates and a divalent cation (Table 2). Magnesium at 10 mM was more active than manganese and incorporation was apparently insensitive to the presence of actinomycin D. The product was sensitive to RNase and incorporation of [^{3}H]UTP was linear for at least 60 min. These conditions are similar to those for VSV virion-associated RNA polymerase, but considerable time and effort will be required to characterize the polymerase activity induced by rabies virus because of its instability (even at 0 C) after sucrose sedimentation, and because of its low activity.

Purified virions of rabies virus lack detectable virion transcriptase in vitro, but carry

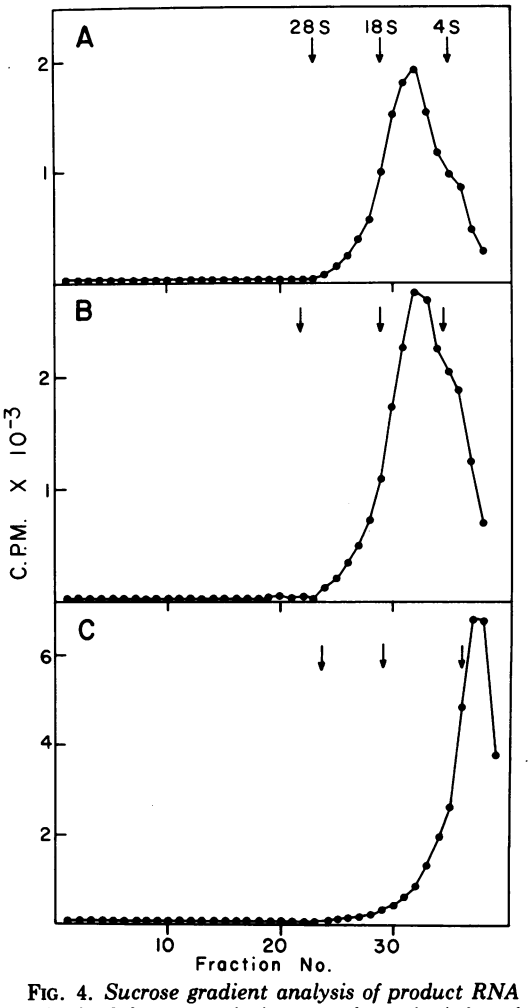

FIG. 4. *Sucrose gradient analysis of product RNA synthesized by transcriptive complexes in infected cells. [³H]uridine-labeled RNA synthesized in vitro by various complexes at 11 h postinfection was extracted with phenol-chloroform (1:1), ethanol precipitated with carrier RNA, and resuspended in 0.3 ml of 0.01 M Tris. Samples were boiled for 10 s and layered on linear 5 to 20% sucrose gradients in 0.1 M NaCl, 0.5 mM EDTA, and 50 mM Tris-hydrochloride, pH 7.6. Gradients were spun at 49,000 rpm for 180 min in a Spinco SW50L centrifuge at 4 C. Ten-drop fractions were spotted onto Whatman DEAE-cellulose, washed, and counted as described (4). Arrows indicate positions of ¹⁴C-labeled HeLa ribosomal RNA. (A) RNA product synthesized by the 175S complex from B virion-infected cells. (B) RNA product synthesized by the 110S complex from B virion-infected cells. (C) RNA product synthesized by the 110S complex of cells infected with B virions at an MOI of 200 and T particles at an MOI of approximately 50. (The number of physical T particles is calculated from a determination of the amount of protein in purified T particle pools, and employing a figure of 30% as the relative mass of these wild-type T particles as compared to B virions.)*

out primary transcription in infected cells. Sokol et al. (17) reported that purified rabies virions lack detectable virion transcriptase, and Table 3 confirms this. We have looked for virion polymerase under a variety of conditions without success. Conditions we have tried included addition of infected and uninfected cell cytoplasm and nucleoplasm to purified rabies virions, trypsinization, addition of UV-inactivated VSV, variations in incubation temperature from 20 to 39 C, and addition of various levels of different divalent cations. No incorporation of [³H]UTP above control levels was detected in any case, and no reverse transcriptase activity could be found associated with rabies virions.

Because of the above results we felt that it was important to rule out the possibility that rabies virus might be a positive strand virus with infectious RNA. Therefore, we attempted to extract infectious RNA from purified virions (0.6 μg of rabies virus RNA was adsorbed to 2×10^6 BHK_{21} cells in the presence of 100 μg of DEAE-dextran per ml for 30 min). No virus plaques and no infectious virus were produced under these conditions where poliovirus RNA was optimally infectious.

Finally, we examined whether purified rabies virions are capable of carrying out primary

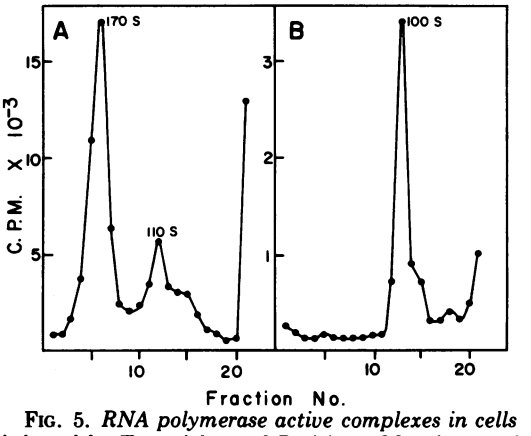

FIG. 5. *RNA polymerase active complexes in cells infected by T particles and B virions. Monolayers of BHK_{21} cells were infected with a mixture of infectious B virions and defective T particles. Cytoplasmic extracts were prepared at about 11 h postinfection and analyzed for RNA polymerase-active complexes as described in Materials and Methods with an incubation time of 90 min. (A) Cytoplasms from cells producing about two-thirds B virions and one-third defective T particles (input MOI of B virions = 200, input MOI of T particles = approximately 5). (B) Cytoplasms from cells producing predominantly defective T particles (input MOI of B virions = 200, input MOI of T particles = approximately 50). T particle multiplicities were estimated as for Fig. 4.*

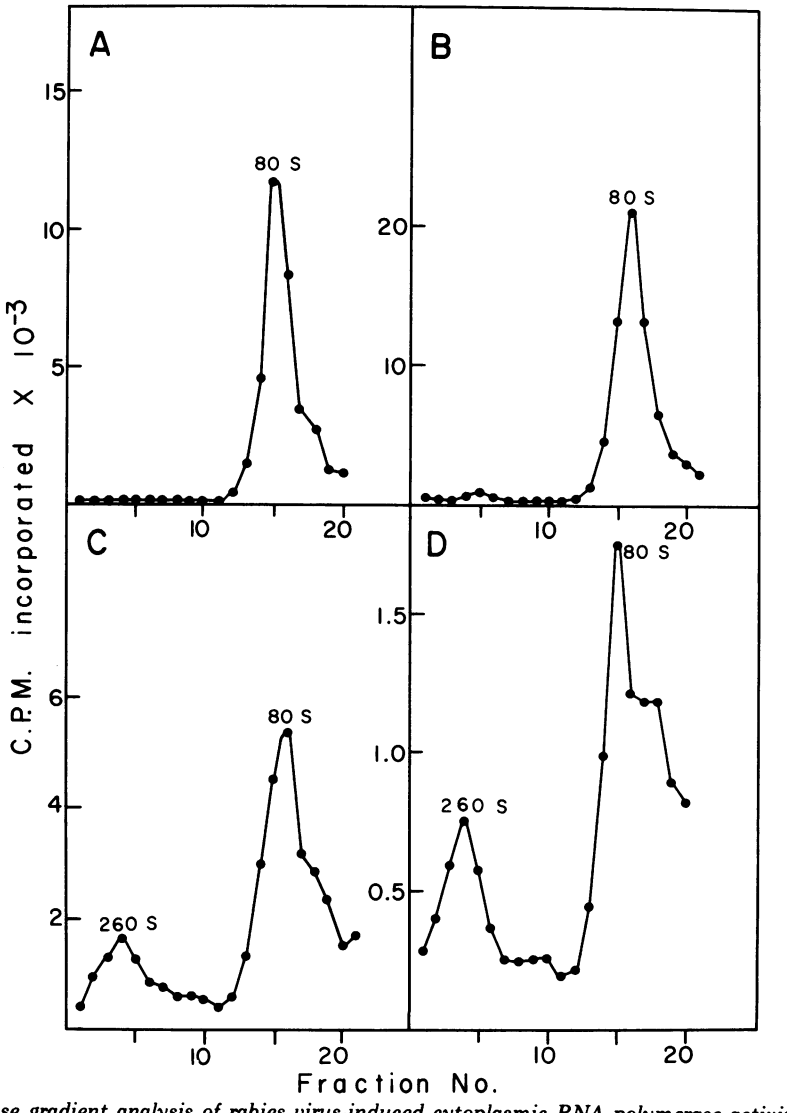

FIG. 6. *Sucrose gradient analysis of rabies virus-induced cytoplasmic RNA polymerase activity. Cytoplasmic extracts of rabies-infected and mock-infected BHK_{21} cells were prepared as described for Fig 1. Isolation and assay of RNA polymerase-active complexes were as described in Materials and Methods with an incubation time of 3 h. (A) Uninfected control 72 h after mock infection; (B) 48 h postinfection with rabies; (C) 72 h postinfection; (D) 84 h postinfection.*

transcription when they infect cells pretreated with actinomycin D and cycloheximide (to prevent cellular synthesis and virus directed protein synthesis as originally described by Marcus for VSV; Science, 174:593–598, 1971). Table 4 shows that purified rabies virions can carry out primary transcription in infected cells. Presumably the natural process of infection of cells is providing some condition or component that is lacking in our in vitro transcriptase assay. However, it is not some component which can be extracted and added to stimulate in vitro virion transcription by rabies since no in vitro virion polymerase can be detected in the presence of added cytoplasm or nucleoplasm from normal or infected cells.

RNA polymerase complexes from carrier cells persistently, noncytopathically infected with VSV. Wagner et al. (22) reported an L cell carrier culture of VSV in 1963. We have recently established a persistently infected BHK_{21} carrier culture of VSV and shown that T particles maintain the balanced carrier state (J. J. Holland and L. P. Villarreal, Proc. Nat. Acad.

TABLE 2. *Characterization of rabies-induced RNA polymerase from infected cells*[a]

Reaction conditions	[^{3}H]UTP incorporation (pmol per 40 μliters per h)
Complete (high specific activity)	39.1
-ATP	5.1
-GTP	11.5
-CTP	4.5
-ATP, GTP, CTP	3.6
1 mM $MgCl_2$	1.3
5 mM $MgCl_2$	43.5
10 mM $MgCl_2$	47.4
1 mM $MnCl_2$	18.2
2 mM $MnCl_2$	22.5
5 mM $MnCl_2$	4.2
Complete (low specific activity) 28 C	135
37 C	100
+50 μg of actinomycin D per ml	140

[a] Rabies RNA polymerase active complex was isolated from the 260*S* region of a sucrose gradient prepared as described in Fig. 6. A 50-μliter amount of this material was mixed with an equal volume of polymerase cocktail to give a final concentration of 0.06 M NaCl, 0.04 M KCl, 50 mM Tris-hydrochloride, pH 7.6, 5 mM $MgCl_2$, 1 mM each ATP, CTP, and GTP, 0.1% β-mercaptoethanol. High specific activity assays were done with 50 μCi/ml of 14 Ci of [^{3}H]UTP per mmol. Low specific activity assays were done with 50 μCi of [^{3}H]UTP per ml and 0.05 mM unlabeled UTP. Modifications to the above concentrations were as described in the table. The reaction mixtures were incubated at 28 C (unless indicated otherwise) for 1 h and then stopped by spotting 40 μliters onto a DEAE-cellulose disk, washing, and counting as described (4).

Sci., in press). Every cell continuously produces small numbers of B virions and T particles. Cell extracts prepared from these carrier cells maintained at 37.5 C (Fig. 7) exhibited two very small peaks of virus-induced polymerase. At 31 C, however, the carrier cultures produce more virus and enter a crisis with cytopathic effect and considerable cell death within several days. Cytoplasmic extracts from cells in crisis at 31 C (Fig. 7A) exhibited two very small peaks of virus-induced polymerase activity. A structure sedimenting at about 175*S* contained approximately one-third of the activity and a structure sedimenting at 120 to 130*S* contained approximately two-thirds of the activity. We have not yet characterized the RNA synthesized by these two structures because the low levels of polymerase activity provide very little product. However, it is clear that persistently infected carrier cells are comparable to rabies virus-infected cells in exhibiting only low levels of viral transcribing complexes as compared to virulent VSV infection.

Viral proteins associated with polymerase complexes from cells infected with VSV B virions. Finally, we examined the structural composition of polymerase complexes from VSV-infected cells. We labeled cells with ^{3}H amino acids during infection, then isolated labeled structures from those regions of the sucrose gradients where active polymerase complexes were found in matched gradients from the same centrifugation run. Figure 8 shows the results of a typical experiment. Figure 8A shows the distribution of active polymerase complex in an NP-40-treated cytoplasmic extract of VSV infected cells. Figure 8B shows the acrylamide gel pattern of labeled proteins associated with the 170*S* polymerase complex. Note that the bulk of the protein is nucleocapsid N protein of VSV (23), and that there are significant quantities of L protein and NS protein, but only traces of the two viral membrane proteins (M and G) can be detected. This provides further

TABLE 3. *Assay for RNA and DNA polymerase activity associated with rabies virions*[a]

RNA transcriptase assay	Incorporated (counts/min)	
	[^{3}H]UTP	[^{3}H]TTP
VSV	39,000	
UV-irradiated VSV	182	
Rabies	182	100
Rabies + cytoplasmic extract	50	100
Rabies + UV-irradiated VSV	185	
Rabies at 38 C	100	
Rabies + 2 mM $MnCl_2$	30	
Rabies + 0.05 μg of trypsin per ml	121	
Rabies + 0.05 μg of chymotrypsin per ml	113	

[a] Purified virions of rabies virus were assayed for transcriptase activity at concentrations between 50 to 200 μg/ml, using RNA polymerase assay conditions as described in Materials and Methods. Virions of VSV were assayed at a concentration of 120 μg/ml. UV irradiation was performed with a UV sterilizing lamp (G8T5-General Electric) at a distance of 5 cm from the lamp in small nitrocellulose cups for 6 min. Cytoplasmic extracts were prepared as described in Fig. 1 and used at an equal volume to the virus. Conditions for reverse transcriptase assay were as follows: 0.05% NP-40, 7 mM Mg acetate, 20 mM Tris-hydrochloride, pH 8.0, 0.1 M NaCl, 0.1% β-mercaptoethanol; 80 μCi of [^{3}H]TTP per ml (46 Ci/mmol from Schwartz); 0.05 mM unlabeled TTP, 1 mM each of dATP, dCTP, and dGTP, 37 C. Counts per minute given indicate insoluble radioactivity per 40 μliters after 3 h of incubation.

TABLE 4. *Primary transcription by purified rabies B virions infecting BHK_{21} cells pretreated with cycloheximide and actinomycin D*[a]

Infection of cells	[^{3}H]uridine (counts/min) incorporated per h	
	Expt 1	Expt 2
None (mock-infected control)	4,500	2,200
VSV B virions	17,000	
Rabies B virions	60,400	8,050

[a] BHK_{21} cell monolayers containing 10^6 cells were preincubated for 30 min at 37 C in MEM containing 2 μg of actinomycin D per ml; then 50 μCi of [^{3}H]uridine per ml (NEN 5 mCi/0.45 mg) was added and preincubation was continued for an additional 30 min at 37 C, all medium was removed, and purified virus was added and allowed to adsorb for 30 min at 33 C in 0.3 ml of MEM containing 2 μg of actinomycin D per ml, 50 μg of cycloheximide per ml, and 50 μCi of [^{3}H]uridine per ml. After virus attachment, 1.5 ml of MEM containing 2 μg of actinomycin D per ml, 50 μg of cycloheximide per ml, and 50 μCi of [^{3}H]uridine per ml was added and incubation was continued for 1 h at 33 C, before the monolayers were extracted with phenol-chloroform (1:1) and the RNA was trichloroacetic acid-precipitated and counted.

evidence that the polymerase complexes are subviral structures. Nearly identical viral protein patterns and ratios were obtained from the 200S polymerase complexes of this experiment (data not shown) so the differing sedimentation rates of these complexes are probably due to nucleocapsid configurational changes. Note also that NP-40 was employed during cell extraction to minimize membrane binding and aggregation of polymerase complexes in this experiment. The presence of NP-40 regularly results in isolation of the larger (170 to 200S) polymerase structures at early and late times postinfection. The reason for this is not obvious.

DISCUSSION

The strong affinity of the cellular poly(U) polymerase for the intact 80S ribosome suggests that its association with the ribosome may not be an artifact, and that it might be involved in some aspect of ribosome function. This ribosomal poly(U) polymerase has been observed in several other cell lines, although the amount of activity present varies considerably (unpublished observations). This cellular polymerase provided a useful marker in our studies but no function has yet been assigned to it (5, 24–26).

In BHK_{21} cells infected with virulent VSV the poly(U) polymerase activity is a relatively minor peak, with most of the RNA polymerase activity residing in virus-induced structures. These polymerase-containing structures probably represent different stages in viral ribonucleoprotein and virion assembly since they contain the nucleocapsid N protein (23) and L and NS proteins (Fig. 8B). Since polymerase complexes of different sedimentation rate contain the same viral proteins in nearly identical ratios they probably represent different degrees of "compactness" of viral nucleoprotein (i.e., ranging from random coil to various degrees of "bullet" assembly).

It is interesting that the presence of T particles alters the pattern of virus-induced polymerase in infected cells. Cells producing approximately two-thirds B virions and one-third T particles show about 60 to 70% of their RNA polymerase activity at 175S and about 30 to 40% at 100 to 110S. Cells producing predominantly T particles exhibit only the 100 to 110S polymerase activity. This activity at 100 to 110S is different than the 100 to 110S activity in cells producing only B virions since the former synthesize only 2S oligonucleotide fragments, and the latter synthesize the usual sizes (10) of VSV mRNA.

BHK_{21} cells infected with a high multiplicity of T particles and B virions synthesize RNA in vivo that is mainly the size of T particle RNA (14). Thus cells producing T particles apparently express more replicase activity (13) than transcriptase activity, yet when the synthetically active complexes are examined in vitro the product is very small. Possibly the 110S com-

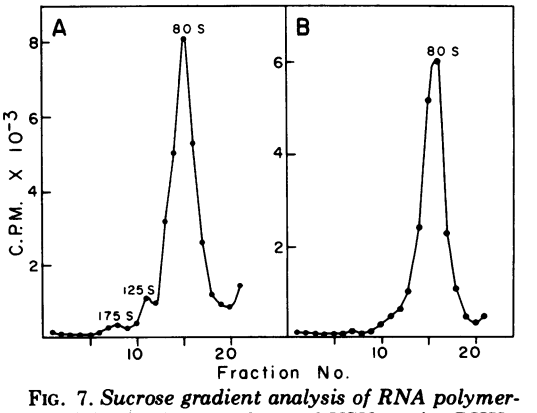

FIG. 7. *Sucrose gradient analysis of RNA polymerase activity in the cytoplasm of VSV carrier BHK_{21} cells. Cytoplasmic extracts of VSV carrier BHK_{21} cells were prepared as described in Fig. 1. Isolation and assay of RNA polymerase-active complexes were as described in Materials and Methods with an incubation time of 90 min. (A) Cytoplasms from cells grown at 31 C. (B) Cytoplasms from cells grown at 37.5 C.*

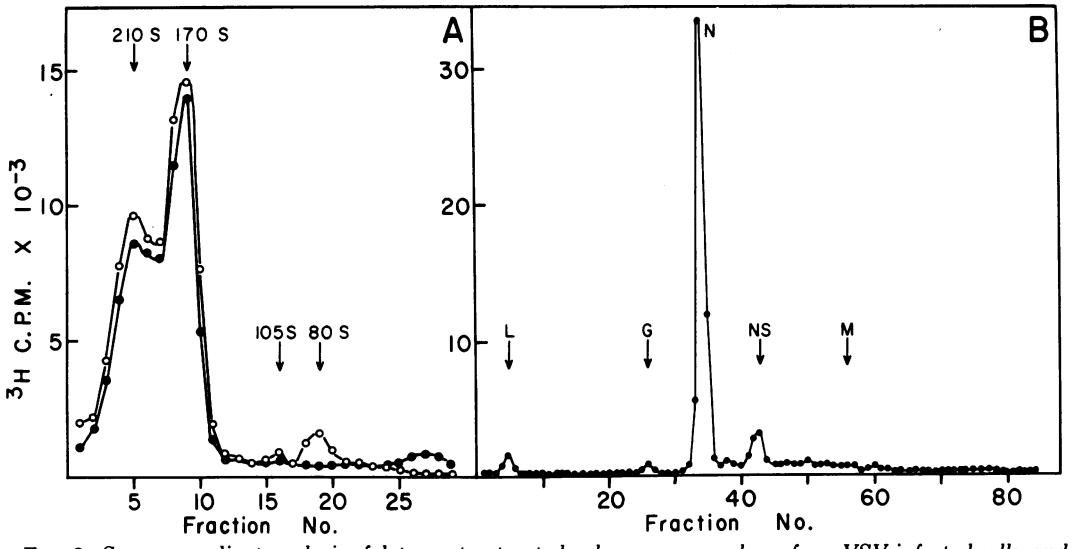

FIG. 8. *Sucrose gradient analysis of detergent-extracted polymerase complexes from VSV-infected cells, and electrophoretic analysis of the viral proteins associated with the 170S complex. (A) Cytoplasmic extracts of BHK_{21} cells infected for 6.5 h with VSV (MOI = 200) were prepared and analyzed for polymerase activity in vitro as for Fig. 1 except that 0.5% NP-40 was added to the cells immediately before disruption, and 15-drop fractions were collected from the 5 to 20% sucrose gradient after centrifugation for 25 min at 4 C in a Spinco SW 50.1 rotor at 49,000 rpm. Symbols: ○, [^{3}H]UTP incorporation; ●, [^{3}H]GTP incorporation during 70-min incubation of each fraction in the transcriptase cocktail. (B) Polyacrylamide gel electropherogram in SDS of ^{3}H amino-acid-labeled proteins associated with the 170S complexes from a matched sucrose gradient identical to A above, but from VSV-infected BHK_{21} cells labeled between 3 and 6.5 h postinfection with [^{3}H]-valine, tyrosine, and phenylalanine before disruption in the presence of 0.5% NP-40 and fractionation on a sucrose gradient as for A.*

plex from cells producing T particles is expressing an abortive replicase activity (13) under conditions which are not optimal for synthesis of full-sized products. In any case, it is obvious that in vitro we cannot yet detect replicase activity for either B virions or T particle RNA.

We have confirmed the lack of virion transcriptase in rabies virions (17) despite many attempts under a variety of conditions. Yet if rabies virus has negative strand RNA in the virion, a virion transcriptase seems mandatory (2). Rabies virions do induce primary transcription in infected cells in vivo, and they induce a sedimenting polymerase complex with detectable in vitro activity. Further characterization of the rabies-induced polymerase complex from infected cells might provide insight into the differences between VSV and rabies in vitro transcription systems.

Finally, it is interesting that the relatively noncytocidal infection of BHK_{21} by rabies virus resembles persistent noncytocidal VSV infection of carrier cultures in exhibiting extremely low levels of polymerase activity. Both lack the high levels of transcriptase activity seen in virulent infection by VSV (compare Fig. 6 and 7 with Fig. 3) and it appears that long T particles suppress transcription in persistent noncytocidal infection of carrier cells (J. J. Holland and L. P. Villarreal, Proc. Nat. Acad. Sci., in press). Further study of these persistent carrier cultures of VSV and of noncytocidal rabies virus infection should provide evidence whether similar regulatory mechanisms are repressing the rate of transcription in both cases.

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