JOURNAL OF VIROLOGY, Nov. 1987, p. 3381–3387
0022-538X/87/113381-07$02.00/0

Vol. 61, No. 11

Characterization of In Vitro Transcription and Transcriptional Products of Measles Virus

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Received 24 February 1987/Accepted 22 July 1987

Ribonucleoprotein complexes isolated from measles virus-infected HeLa cells contained an RNA-dependent RNA polymerase activity that catalyzed the incorporation of ribonucleotides into ribonucleic acid. The ribonucleoprotein complexes were composed of measles virus nucleoprotein, phosphoprotein, and a large protein, as well as viral RNA. The kinetics of RNA synthesis at different temperatures, time intervals, and protein, ribonucleotide, and mono- and divalent cation concentrations were analyzed. Enzyme activity was maximum at 4 h at 25°C in the presence of 100 mM Na^+–2.5 mM Mg^{2+}–1 mM ribonucleotides. Actinomycin D and α-amanitin had no effect on the enzyme activity. Addition of cytoplasmic extracts from uninfected HeLa cells to the reaction mixture did not increase the incorporation of ribonucleotides into RNA. The in vitro synthesized RNAs were characterized by slot blot analysis and quantitated by densitometer scanning. All mRNAs coding for the structural proteins of measles virus were synthesized. Nucleoprotein RNA was the most abundant species made, followed by phosphoprotein, hemagglutinin, fusion protein, matrix protein, and large-protein RNAs. The system described here resulted in the first efficient transcription of measles virus RNA and analysis of products.

Measles virus, a negative-strand RNA virus of the paramyxovirus family, can cause three distinct syndromes in humans. First, infection by measles virus can result in an acute respiratory tract infection leading to systemic spread to other parts of the body. As an immune response is mounted, virus is rapidly cleared from the body and the individual usually recovers. Second, 5 to 7 days after the appearance of the characteristic rash, measles virus can occasionally induce an autoimmune-like condition involving the central nervous system known as postinfectious encephalomyelitis. However, virus is rarely isolated from the central nervous system. Peripheral blood mononuclear cells from these patients proliferate in response to the presence of myelin components, as well as measles virus antigens (25). Third, on rare instances, measles virus can persist in the central nervous system and lymphoid cells in humans. This syndrome is known as subacute sclerosing panencephalitis. Virus persists despite the presence of high titers of antiviral antibody and competent immune cells. Neurologic changes are associated with the presence of virus. Infection of this type is usually fatal. Virus can often be recovered from central nervous system tissue and lymphoid cells by cocultivation techniques (3, 22, 29). Recently, viral nucleic acid has been demonstrated in brains and peripheral blood mononuclear cells from subacute sclerosing panencephalitis patients by in situ hybridization even though no viral antigen or infectious virus could be demonstrated (4, 5, 13, 19).

Measles virus nucleocapsids contain an RNA-dependent RNA polymerase that transcribes in vivo at least seven mRNAs from the negative-strand genome (16, 27, 36, 39). These ribonucleoprotein (RNP) complexes are composed of the single-strand genome RNA and at least three virally encoded proteins: nucleocapsid (NP), phosphoprotein (P), and large (L) protein. Although an in vitro transcription system for measles virus has been described, it used RNP complexes from purified virions or detergent-treated virus (34). We developed a more efficient in vitro transcription system by using RNP complexes isolated from infected cells. In this paper, we describe the properties and substrate requirements for the transcription of measles virus RNA and the first analysis of the synthesized products.

MATERIALS AND METHODS

Virus and cells. The Edmonston strain of measles virus was used for all experiments. Virus stocks were grown in Vero cells as previously described (16). These high-titer stocks were then used to infect HeLa cells. HeLa cells were grown in suspension as previously described (17) and infected at a multiplicity of 2 to 3 PFU per cell.

Preparation of measles virus cellular RNP complexes. RNP complexes were prepared by the method of Hill and Summers (21), with the following modifications. HeLa cells infected with measles virus were collected 48 h after infection by centrifugation at 1,000 × *g* for 5 min and washed with cold phosphate-buffered saline. The pelleted cell volume was determined, and cells were suspended in an equal volume of lysis buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4], 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol). The cells were allowed to swell for 5 min on ice and then disrupted with 30 strokes of a Dounce homogenizer. The homogenate was clarified by centrifugation at 8,000 rpm in a Sorvall SS.34 rotor for 15 min at 4°C. The supernatant fluid was made 0.5 M NaCl and 1% Triton X-100 and centrifuged through 2.5 ml of 50% glycerol in the lysis buffer (described above) for 2 h at 45,000 rpm in a Beckman SW50.1 rotor at 4°C. The pellet containing RNP complexes was suspended in 2× transcription buffer (100 mM HEPES [pH 7.9], 10 mM dithiothreitol, 200 mM NaCl, 5 mM $MgCl_2$, 0.1% Triton X-100). The RNP complexes were frozen immediately in small portions at −70°C. For determination of the dependence of RNA-dependent RNA polymerase activity on mono- or divalent cations, the RNP complexes were dissolved in 2× transcription buffer

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which did not contain Na^+ or Mg^{2+}. The cations were added separately at different concentrations over the described ranges in the reaction mixtures.

Protein analysis. The RNP complexes were prepared as described above at 48 h postinfection from measles virus-infected HeLa cells labeled with [^{35}S]methionine (1,200 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) 4 h prior to harvest. The proteins were analyzed by sodium dodecyl sulfate (SDS)–10.5% polyacrylamide gel electrophoresis. Gels were stained with Coomassie brilliant blue dye, destained, and dried on filter paper. X-ray film (Kodak XRP-1) was exposed to the dried gel and then developed after 2 days of exposure.

RNA polymerase assay. The RNA polymerase assay was performed in a reaction mixture (10 μl) containing 50 mM HEPES (pH 7.9), 5 mM dithiothreitol, 0.05% Triton X-100, 100 mM NaCl, 2.5 mM $MgCl_2$, 1 mM each ATP, CTP, and GTP, 50 μM UTP, 5 μCi of [α-^{32}P]UTP (3,000 Ci/mmol, New England Nuclear Corp., Boston, Mass.), human placental RNase inhibitor (Bethesda Research Laboratories, Gaithersburg, Md.), and 20 to 30 μg of RNP complexes. The RNase inhibitor was included to minimize possible degradation of RNA transcripts. The mixture was then incubated for 4 h at 25°C. The reaction was stopped by addition of 10 μg of *Saccharomyces cerevisiae* tRNA (10 mg/ml) and 0.5 ml of cold 10% trichloroacetic acid (TCA). The acid-insoluble material was collected on glass fiber filters (GN-6; Gelman Sciences, Inc., Ann Arbor, Mich.) and washed extensively with ice-cold 5% TCA–cold ethanol. The filters were dried, and incorporation was measured by liquid scintillation counting. To test the effect of actinomycin D (Calbiochem-Behring, La Jolla, Calif.) or α-amanitin, (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), the reagents were added directly to the in vitro reaction mixtures. Actinomycin D was added at a concentration of 20 μg/ml, and α-amanitin was at 2 μg/ml. Incorporation of [α-^{32}P]UTP in an acid-insoluble form was measured by TCA precipitation.

For analysis of in vitro transcripts on gels, the reaction was stopped by addition of 180 μl of 1× STE buffer (0.15 M NaCl, 5 mM EDTA, 50 mM Tris [pH 8.0]) and 20 μg of *S. cerevisiae* tRNA. The reaction mixture was extracted with an equal volume of phenol-chloroform (1:1). The aqueous layer was made 0.3 M by addition of 3 M sodium acetate, and 2.5 volumes of cold ethanol was added to precipitate RNA. The precipitates were collected by centrifugation and washed once with 70% ethanol. Samples were dried and treated with glyoxal (see agarose gel electrophoresis).

Agarose gel electrophoresis. To analyze RNA under denaturing conditions, samples were incubated in 1 M glyoxal–50% (vol/vol) dimethyl sulfoxide–10 mM sodium phosphate buffer (pH 7.0) for 1 h at 50°C and for 1 h at room temperature. The samples were made to 5% glycerol–0.025% bromophenol blue–0.025% xylene cyanol and then electrophoresed in a horizontal 1.2% agarose gel. The gel running buffer was 10 mM sodium phosphate (pH 7.0), which was recirculated constantly (38). For autoradiography, the gels were dried and exposed to Kodak XRP-1 film with intensifying screens (Du Pont Co., Wilmington, Del.).

Slot blot analysis. Measles cDNA clones NP, P, M (membrane or matrix) protein, L protein, HA (hemagglutinin), F (fusion) protein, and three other unrelated cDNA clones (Theiler's virus, mumps virus, and rubella virus) were used in slot blot analysis (see Acknowledgments). All of the cDNAs were cloned at the *Pst*I site of pBR322. The plasmid DNA was purified by the CsCl-ethidium bromide gradient centrifugation method. The cDNAs (2 μg per sample) were denatured by addition of 0.1 volume of 3 N NaOH and incubated at 60°C for 1 h. The samples were neutralized by addition of an equal volume of 2 M ammonium acetate (pH 7.0). The samples were blotted on nitrocellulose in a Schleicher & Schuell, Inc. (Keene, N.H.), slot blot apparatus. The nitrocellulose was baked at 80°C in a vacuum for 30 min. The blot (nitrocellulose) was prehybridized in hybridization buffer (50% formamide, 50 mM phosphate buffer [pH 6.4], 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 5× Denhardt solution, 0.1% SDS, 100 μg of denatured salmon sperm DNA per ml) at 42°C overnight. A reaction mixture similar to the one described above (see RNA polymerase assay) was used to prepare ^{32}P-labeled in vitro RNA transcripts, except that the total volume was doubled. After incubation, the reaction mixture was extracted with a phenol-chloroform mixture and ethanol precipitated. The ethanol precipitate was solubilized in 10 mM Tris (pH 7.5)–1 mM EDTA buffer, boiled for 1 min, and used as a probe. The slot was incubated for 48 h with the probe in hybridization buffer. The blot was washed three times with 1× SSC–0.1% SDS at room temperature for 5 min each time, followed by two washes in 0.1× SSC–0.1% SDS at 50°C for 15 min each time. The blot was autoradiographed at −70°C with an intensifying screen. Densitometer scanning of the slot blot autoradiograph was done in an LKB Ultrascan XL laser densitometer.

RESULTS

Analysis of RNP. Radiolabeled RNP complexes and supernatant fluid from measles virus-infected HeLa cell extract (material before pelleting through 50% glycerol) were analyzed by SDS-polyacrylamide gel electrophoresis followed

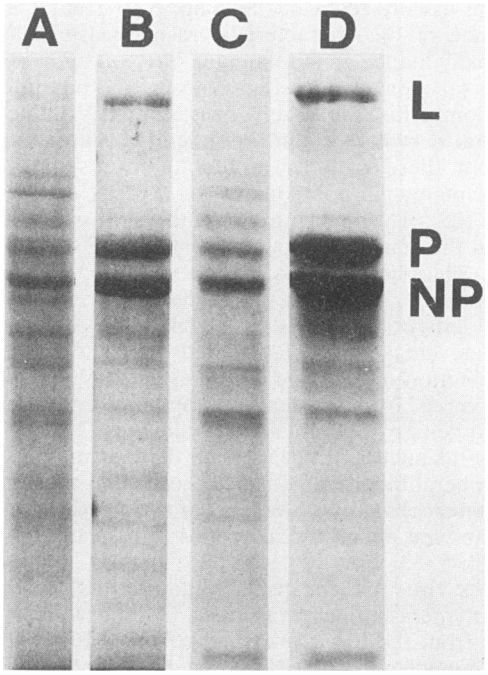

FIG. 1. Coomassie blue-stained pattern (lanes A and B) and autoradiogram of a 10% polyacrylamide gel of [^{35}S]methionine-radiolabeled proteins (lanes C and D). Lanes: A and C, proteins present in the supernatant after the cells were lysed and the debris was removed by low-speed centrifugation; B and D, cellular RNP isolated by centrifugation of the supernatant through a 50% glycerol cushion. A total of 30 μl of each sample was loaded on the gel.

by autoradiography (Fig. 1). Although a number of proteins (both cellular and viral) were present in the supernatant of infected-cell extracts (lane A) or RNP material pelleted from the cell lysate (lane B), most of the material in the RNP complexes consisted of the L protein, P, and NP. Autoradiography of the gel showed the same results for [^{35}S]methionine-labeled proteins. By this method, L protein could clearly be delineated (lanes B and D). Therefore, the RNP complexes were highly enriched by a single centrifugation through glycerol.

Requirement for virus polymerase activity. Addition of RNP complexes (see Materials and Methods) to a reaction mixture containing 1 mM ribonucleotide triphosphates and 5 μCi of [α^{32}P]UTP resulted in incorporation of [^{32}P]UTP into acid-insoluble material. RNA synthesis was found to depend on a number of parameters.

(i) Dependence on temperature and time. To characterize the optimal temperature for the reaction, the transcription reaction was performed at three different temperatures, 22, 25, and 31°C, over a period of 1 to 6 h. RNA synthesis was measured by incorporation of [^{32}P]UMP into TCA-precipitated materials. For all of the temperatures tested, the RNA synthesis kinetics were linear over the first 4 h (Fig. 2), with the reaction at 25°C being the maximum. After 4 h, reactions at all temperatures plateaued.

(ii) Dependence on protein concentration. A relationship between the amount of RNP present in the reaction mixture and RNA synthesis was observed. Incorporation of [^{32}P]UMP was 1.8 pmol per 4 h at 8 μg per reaction and 6.2 pmol of [^{32}P]UMP at 20 μg per reaction. Higher protein concentrations (32 μg per reaction) led to the incorporation of 10.9 pmol of [^{32}P]UMP (Table 1). Thus, the rate of reaction was roughly proportional to protein concentrations.

TABLE 1. Properties of measles virus DNA polymerase

Addition to reaction system[a]	[^{32}P]UMP incorporation into acid-insoluble material (pmol/4h)
Ribonucleoside triphosphates	
1 mM	12.3
2.5 mM	5.6
5 mM	0
Actinomycin D (20 μg/ml)	10.2
α-Amanitin (2 μg/ml)	11.9
Equal vol of infected cell extract	9.4
Uninfected cell extract (50 μg)	0.8
RNP	
8 μg	1.8
20 μg	6.2
32 μg	10.9

[a] The complete system contained 50 mM HEPES (pH 7.9), 100 mM $NaCl_2$, 0.05% Triton X-100, 5 mM dithiothreitol, 1 mM each ATP, CTP, and GTP, 50 μM UTP, and 5 μCi of [α-^{32}P]UTP (3,000 Ci/mmol; New England Nuclear) in a reaction volume of 10 μl along with 30 μg of RNP complexes.

(iii) Dependence on monovalent cation concentration. To determine the concentrations of monovalent cations required for in vitro synthesis of RNAs, varied amounts of NaCl and KCl were added to reaction mixtures. There was incorporation of 2 pmol of [^{32}P]UMP into TCA-precipitable materials when no NaCl or KCl was included in the reaction mixture (Fig. 3). For both NaCl and KCl, as the concentration of monovalent ions increased, there was a steady increase of incorporation into TCA-precipitable materials until 100 mM, and then incorporation decreased. The difference in incorporation between 0 to 100 mM concentrations was approximately threefold. No significant difference in incorporation was detected between NaCl and KCl as sources of monovalent cations.

(iv) Dependence on divalent cation concentration. It has been reported for a number of viruses that Mg^{2+} is a

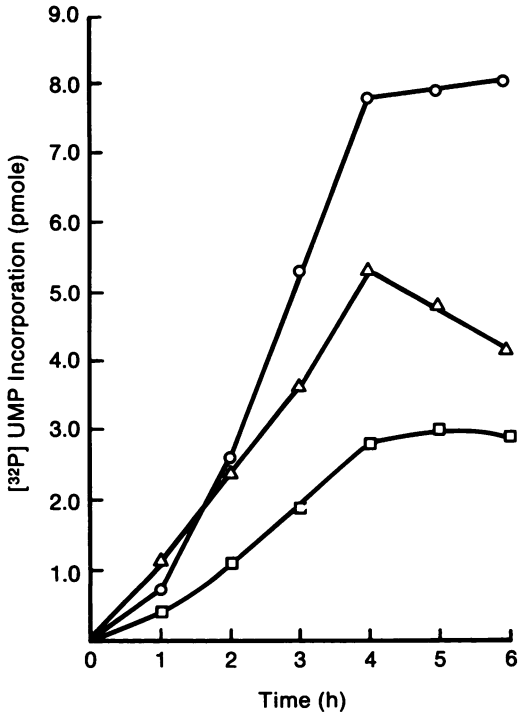

FIG. 2. Time course of RNA synthesis. Standard reaction mixtures containing RNP complexes were incubated at 22 (□), 25 (○), or 31°C (△) for the indicated times. The reaction mixtures were TCA precipitated and counted in a scintillation counter.

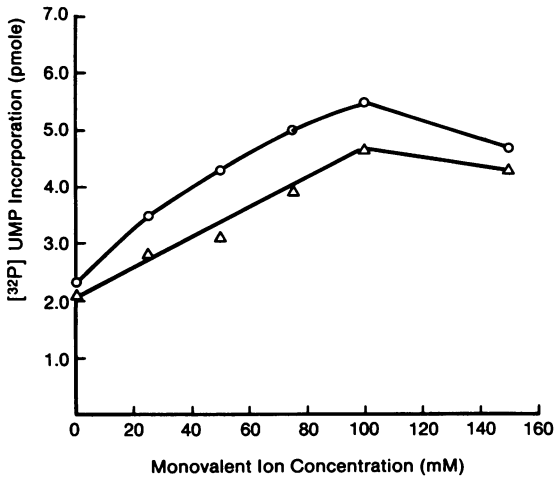

FIG. 3. Dependence of RNA synthesis on monovalent cations. Standard reaction mixtures containing the indicated concentrations of NaCl (○) or KCl (△) were incubated at 25°C for 4 h. The samples were TCA precipitated and counted in a scintillation counter.

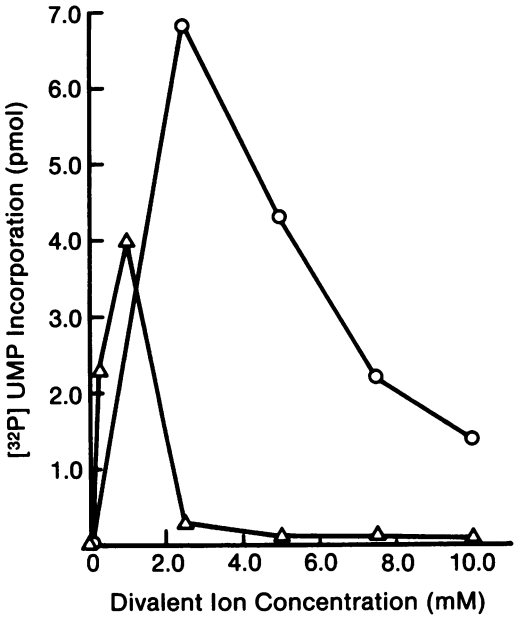

FIG. 4. Dependence of RNA synthesis on divalent cations. Standard reaction mixtures containing the indicated concentrations of $MgCl_2$ (○) or $MnCl_2$ (△) were incubated at 25°C for 4 h. The samples were TCA precipitated and counted in a scintillation counter.

requirement for in vitro transcription (6, 24). In the case of measles virus, no incorporation occurred in the absence of exogenous Mg^{2+} added to the reaction mixture. Incorporation was maximal at 2.5 mM and then rapidly decreased at higher concentrations (Fig. 4). Lower concentrations of Mn^{2+} than Mg^{2+} were needed for polymerase activity. The incorporation of [^{32}P]UMP was 4.0 pmol in the presence of 1 mM Mn^{2+}, compared with 6.8 pmol at 2.5 mM Mg^{2+} (Fig. 4). Lower concentrations of Mg^{2+} (less than 2.5 mM) did not result in increased incorporation over that observed at 2.5 mM (data not shown). Mn^{2+} concentrations above 3 mM completely inhibited the polymerase activity (Fig. 4).

(v) Dependence on ribonucleotide triphosphates. RNA synthesis required all four triphosphates. The optimal concentration of triphosphates in the reaction mixture was 1 mM. Incorporation into TCA-precipitable material decreased by 50% at 2.5 mM, and no incorporation was noted at concentrations above 5 mM (Table 1).

(vi) Effects of actinomycin D and α-amanitin. Transcription reactions were performed in the presence of actinomycin D (20 μg/ml). At this concentration of actinomycin D, the transcription reaction was not inhibited. Thus, incorporation of nucleotides into RNA was not due to contaminating DNA-dependent RNA polymerase. Also, no decrease of transcription was noted when α-amanitin (2 μg/ml) was added to the reaction mixture (Table 1). α-Amanitin is a potent inhibitor of RNA polymerase II (26). These results indicate that incorporation of [^{32}P]UMP in acid-insoluble precipitate was due to viral transcription and not contaminating cellular polymerases.

(vii) Effect of cellular components. SDS-polyacrylamide gel electrophoresis of RNP complexes isolated from infected HeLa cells identified trace amounts of ^{35}S-labeled cellular proteins (Fig. 1). Coomassie blue staining of the same gel showed that only small amounts of cellular proteins were present and made up less than 5% of the total protein (Fig. 1, lane B). To determine whether these proteins could contribute to the polymerase activity by themselves, preparations were made from uninfected HeLa cells in a manner identical to that used for those prepared from infected cells, i.e., RNP complexes. No incorporation of [^{32}P]UMP was noted when preparations from uninfected cells were used in the transcription reaction (Table 1). Similarly, when cytosol preparations from uninfected cells were added to the reaction mixture containing RNP complexes from infected cells (equal amounts), no increase of incorporation of [^{32}P]UMP into macromolecules was observed.

Analysis of RNA products. RNA synthesized in vitro was analyzed under denaturing conditions by glyoxal-agarose gel electrophoresis. The distribution of radioactive bands in the gels as evidenced by autoradiography indicated the presence of a number of RNA transcripts migrating in the 16S to 35S (as estimated by 18S and 28S markers) size range (Fig. 5). The band at about 22S could represent F protein mRNA (14) or a polycistronic transcript which has been previously reported to be present in measles virus-infected cells (4). In view of the mass estimates of measles virus structural proteins, it was likely that L protein mRNA was specified by a band migrating at about 35S (14, 32). In these experiments, full-length genomic RNA transcripts (50S) were not detected.

To determine whether the measles virus RNA transcripts synthesized in the in vitro transcription system represented RNAs coding for viral structural proteins, slot blot analysis was performed. Purified plasmid DNAs containing cDNA inserts of six structural measles virus genes (NP, P, M, F, HA, and L) were spotted in equal amounts on nitrocellulose paper. As a control, DNAs from cDNA clones of Theiler's virus, mumps virus, and rubella virus were used. In vitro transcripts labeled with [^{32}P]UTP were used as probes. Hybridization reactions were performed. NP RNA was the predominant species (Fig. 6A). P RNA was also produced in large amounts. M, F, and L protein RNAs were synthesized in similar amounts. In several experiments, HA RNA was made in slightly higher amounts than M, F, and L protein

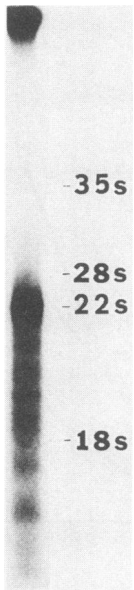

FIG. 5. Electrophoretic analysis of RNA products synthesized in vitro. ^{32}P-labeled RNA was denatured by treatment with glyoxal-dimethyl sulfoxide, electrophoresed on a 1.2% agarose gel, dried, and autoradiographed.

transcripts, and L protein RNA was synthesized the least. The difference in hybridization of the various mRNAs synthesized in vitro with DNAs fixed on nitrocellulose should be representative, since the same amounts of purified cDNA were used for each slot and they exceeded those of the input labeled material. Densitometer scanning (Fig. 6B) of the autoradiograph showed that 68.7% of the transcripts were NP RNA. Of the total transcripts, P represented 23.4%, HA represented 3.0%, F protein was 2.4%, M protein was 1.5%, and L protein was 1.0%. This result indicated that all measles virus structural protein mRNAs were synthesized in the in vitro transcription assay.

DISCUSSION

We demonstrated that in vitro transcription of measles virus RNA from RNP complexes isolated from infected HeLa cells led to the production of viral RNAs representing all of the structural proteins. The RNP complexes were composed of L protein, P, and NP, and when the complexes were added to the reaction mixture, incorporation of radiolabeled monophosphate into TCA-precipitable material occurred.

Earlier, Seifried et al. (34) reported measles virus polymerase activity in vitro when reactions were conducted at 28°C. However, no temperature optimum for the reaction was determined. In our studies, we observed that the polymerase activity of measles virus was temperature dependent. The optimum temperature for our reaction conditions was 25°C. It is interesting that this temperature was lower than the optimal temperatures reported for other negative-strand viral polymerases. Whereas the temperature optimum for vesicular stomatitis virus (VSV) and Sendai virus was reported to be 28°C (1, 37), it was 32°C for Newcastle disease virus (NDV) and 31°C for Kern Canyon virus (1, 23). These in vitro experiments were contrasted by in vivo experiments

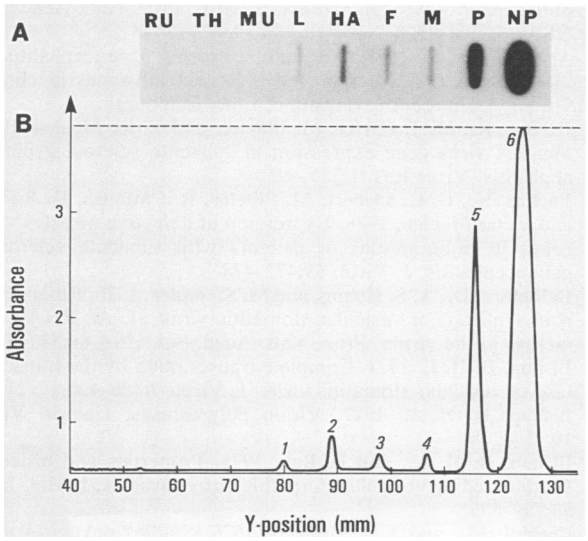

FIG. 6. Slot blot analysis and densitometer scanning of in vitro transcribed measles virus RNA. (A) DNAs (2 μg each) prepared from measles virus NP, P, M, F, HA, and L cDNA clones and also mumps virus, Theiler's virus, and rubella virus cDNA clones were denatured and blotted on nitrocellulose filters. ^{32}P-labeled RNA synthesized in vitro with RNP complex isolated from measles-infected cells was used as a probe. (B) Densitometer scanning of the slot blot was done in an LKB Ultrascan XL laser densitometer. The area under each peak was calculated by the machine.

that demonstrated that the temperature optimal for primary transcription of VSV was 38°C (15).

Our results indicate that the rate of UMP incorporation was proportional to protein concentration (Table 1). Similar results have been reported for NDV, a paramyxovirus similar to measles virus (23). However, Perrault and Kingsbury (30), using purified VSV, reported that the polymerase activity was inhibited at higher virus concentrations. Breindl and Holland (10) confirmed this finding but, in contrast, reported that when the transcribing activity of VSV RNP complexes was tested, the transcription activity increased linearly with increasing template concentrations. Further, it has been reported that virion M protein was responsible for the endogenous inhibition of in vitro RNA synthesis seen at high concentration of VSV (11, 12).

For VSV and Kern Canyon virus in vitro polymerase assays, the monovalent cation concentration requirement was approximately 100 mM and was independent of the cation present (1). For example, at similar monovalent cation concentrations for Na^+, K^+, Li^+, and NH_4^+, all were equally effective for VSV and Kern Canyon virus transcription (1). In contrast, we found that for measles virus there was incorporation of radiolabel into TCA-precipitable material in the absence of exogenously added Na^+. However, the incorporation increased with the addition of Na^+ and was optimal at an added concentration of 100 mM.

The divalent cations most commonly used for the transcription reactions with other paramyxoviruses were Mg^{2+}, Mn^{2+}, or both (8). Others have demonstrated that Mn^{2+} had no effect on the transcription of VSV (1), Sendai virus (33), and NDV (23). However Seifried et al. (34) reported that Mn^{2+} increased the incorporation of nucleotides into RNA for measles virus. In our system, the polymerase activity was stimulated by addition of Mn^{2+} at a concentration of 1 mM or lower. However, the polymerase activity in the presence of Mn^{2+} was not as great as with Mg^{2+}. We found that the optimum added concentration of Mg^{2+} for measles virus transcription was 2.5 mM (Fig. 4). At higher concentrations of Mg^{2+}, the incorporation decreased. The Mg^{2+} concentration for measles virus was in good agreement with those of other viral systems (VSV, Sendai virus, and NDV), whose Mg^{2+} concentration requirements were 3 to 10 mM (8). In a separate experiment, purified measles virions were used in an assay for polymerase activity under similar experimental conditions (unpublished data). The polymerase activity as measured by UMP incorporation showed that the activity obtained with purified virions was 1/10 of that obtained with RNP complexes isolated from infected cells. The activity with virion RNP complexes could be increased twofold by addition of 7.5 mM Co^{2+}. However, with cellular RNP complexes the activity decreased by 60% when Co^{2+} was used instead of Mg^{2+} (unpublished data). For Sendai virus (37) and VSV (Ray, unpublished data), Co^{2+} had no effect on polymerase activity. We would like to point out that the concentration of mono- or divalent cations reported here was the added concentration and not the ultimate concentration, which may vary depending on their presence in cells or lysis buffer.

The optimal concentrations for ribonucleotide triphosphates have been determined for several viral polymerases. At least for VSV, the concentration of triphosphates required to give optimal activity varied depending on which triphosphate was considered. A concentration of 0.5 to 1 mM for each triphosphate was suitable to obtain maximal activity, except for ATP, for which 2 mM or higher was required for efficient VSV transcription (8). Although in our

study we did not analyze the effect of each triphosphate individually, a concentration of 1 mM for the mixture of triphosphates was adequate.

Recently, it has been reported for VSV and Sendai virus that a cellular protein stimulated in vitro transcription reactions (20, 28). Since our measles virus RNP complexes contained trace amounts of cellular proteins, we wanted to see whether the transcription reaction could be enhanced by addition of uninfected cellular extract. When the uninfected extract was added to RNP complex-containing reaction mixtures, no increase in incorporation of [^{32}P]UMP into TCA-precipitable material was noted. Therefore, cellular elements necessary for measles virus transcription must be already associated with the viral RNP complexes and present in low amounts. However, Piwinica-Worms and Keene (31) reported that addition of uninfected cell extracts to an in vitro transcription reaction of VSV increased the production of leader RNA and the disappearance of capped and triphosphate-containing RNA species, although the effect of cell extracts on synthesis of mRNA was not examined. Recently, we have obtained a salt-stripped, microtubule-associated protein preparation from D. Summers (20) and are testing its effects in our systems. Preliminary experiments indicate that the microtubule-associated protein preparation can enhance the incorporation of [^{32}P]UMP into TCA-precipitable material in our system.

In vitro transcription product size analysis by sucrose density gradient centrifugation for VSV (7, 9), NDV (23), Sendai virus (33, 37), and measles virus (34) showed that the transcripts possessed a size range comparable to that of the various mRNA species from infected cells. To our knowledge, nothing has been reported concerning the extent or proportional representation of the various in vitro transcripts. Our report is unique in that it describes both the sizes of mRNAs synthesized in vitro and their relative abundance. Measles virus cDNA corresponding to parts of the genome or mRNA of the virus has become available for the molecular analysis of measles virus and subacute sclerosing panencephalitis virus. By Northern (RNA) blot analysis, it was possible to order the clones according to the pattern of individual gene-specific and readthrough mRNAs. The results indicated that the transcriptional map and the gene order of measles virus was 3′-NP-P+C-M-F-H-L-5′ (14, 32) and was similar to that of Sendai virus (35). From the study of Sendai virus (18, 35) and VSV (2), it has been suggested that the viral mRNAs are transcribed sequentially by viral transcriptase. The mRNA coding for NP, the most abundant viral protein, is the first to be transcribed, and the mRNA coding for L protein, the least abundant viral protein, is the last. By using in vitro transcription with measles virus RNP and slot blot analysis, we obtained results indicating that the transcription of measles virus was similar to those of Sendai virus and VSV. In our experiments, NP mRNA was the most abundant species synthesized during in vitro transcription, followed by P mRNA. mRNA for L protein was made the least. One exception was the amount of mRNA for HA, although downstream it was synthesized more efficiently than M or F protein transcripts (Fig. 6).

Currently, our system does not distinguish between chain initiation and chain elongation during in vitro reaction. In an attempt to determine the presence of small RNA transcripts attached to the RNP complexes, RNA was extracted from RNP complexes and analyzed by Northern blot analysis with [^{32}P]NP cDNA as a probe. Very few small transcripts were detected, indicating the presence of few already initiated chains (unpublished data). Therefore, most of the incorporation of [^{32}P]UTP into transcripts was due to chain initiation.

The system we describe here yields efficient transcription of measles virus RNA. By using this assay, the specific role and interaction of various measles viral proteins essential for transcription and replication can be resolved.

ACKNOWLEDGMENTS

This work was supported by National Multiple Sclerosis Society grants GR NMSS 2029-A and GR NMSS 1780-A and by Public Health Service grants AI0 70007 and NS 12428 from the National Institutes of Health. R.S.F. is a Harry Weaver Scholar of the National Multiple Sclerosis Society.

We thank W. Bellini and S. Rozenblatt, Centers for Disease Control, Atlanta, Ga., for measles virus NP, P, and M protein cDNA clones; B. Blumberg, University of Medicine and Dentistry of New Jersey, Newark, for the measles virus L cDNA clone; H. Lipton, Northwestern University, Chicago, Ill., for the Theiler's virus cDNA clone; S. R. Petteway, Du Pont Co., Wilmington, Del., for the rubella virus cDNA clone; and B. K. Rima, The Queen's University of Belfast, Belfast, U.K., for the mumps virus cDNA clone. We thank Peggy Farness and Susan McClanahan for technical assistance and Diana Ferris and Gail Parish for expert typing of the manuscript. In addition, we acknowledge Joel Gottesfield of Scripps Clinic & Research Foundation for the use of his laser scanner, J. A. Nelson, P. Southern, and L. Whitton of Scripps Clinic & Research Foundation, John Holland of the University of California San Diego, and D. Summer and E. Ehrenfeld of the University of Utah for critical review of the manuscript. We gratefully thank M. B. A. Oldstone for his helpful discussions and continued support.

LITERATURE CITED

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