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Synthesis of Leader RNA and Editing of the P mRNA during Transcription by Purified Measles Virus

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A transcription system with detergent-disrupted purified measles virus was developed. Synthesis of authentic, full-length measles virus N, P, M, and F mRNAs by purified virus occurred as identified by dot-blot hybridization analysis of individual measles virus clones and gel electrophoresis. The relative abundance of the first five viral mRNAs synthesized in vitro decreased significantly with their distance from the 3′ end. The addition of the soluble protein fraction from uninfected A549 cells stimulated overall viral RNA synthesis but did not alter the relative abundance of each of the mRNAs. Measles virus synthesized in vitro a leader RNA of ~55 nucleotides in length, suggesting that like other negative-strand viruses, transcription initiated only at the 3′ end of the genome RNA. Purified measles virus also catalyzed RNA editing during the synthesis of the P mRNA as shown by modified primer extension analysis of the mRNA products and by translation of the modified RNA into the V protein in rabbit reticulocyte lysates. These data suggested that the RNA editing activity was virus encoded.

Measles virus, a member of the paramyxovirus family, contains a nucleocapsid with the single-stranded RNA genome (16 kb) of the negative-sense strand encapsidated in the major nucleocapsid protein N (12, 17, 21). The virion also contains an RNA-dependent RNA polymerase consisting of the viral L and P protein subunits, which catalyze the transcription and replication of the nucleocapsid template. The envelope proteins F, H, and M constitute the remaining structural proteins of the virion (11). In infected cells there are, in addition, two nonstructural viral proteins whose functions are unknown: the C protein, which is synthesized from an overlapping reading frame of the P mRNA (1, 7) and the V protein, which is translated from an edited transcript of the P gene (5).

Transcription of the paramyxovirus genome RNA occurs from the 3′ end to the 5′ end of the genome such that the mRNAs are synthesized sequentially in the order N, P/C, M, F, H, and L (7, 8, 18, 19, 22, 23). Before the synthesis of the N mRNA, Sendai virus and another negative-strand RNA virus, vesicular stomatitis virus, have been shown to synthesize both in vivo and in vitro a short leader RNA (45 to 55 nucleotides) which was initiated precisely at the 3′ end of the genome (10). In contrast, no leader RNA has been detected in measles virus-infected cells (3, 4, 6). Instead, Castaneda and Wong (3, 4) have shown that a polyadenylated leader-N readthrough RNA was present in measles virus-infected cells. This RNA was, however, encapsidated in N protein and was apparently not translated, since it was not associated with polysomes. These observations led to the hypothesis that, unlike other negative-strand viruses, measles virus may have two RNA initiation sites, one directly at the 3′ end for the synthesis of the positive-strand replicative RNA and a second at the beginning of the N gene for productive mRNA synthesis.

An unusual phenomenon, RNA editing, has recently been described for a number of the paramyxoviruses, including simian virus 5, measles, Sendai, and mumps viruses, and parainfluenza virus types 2 and 4 (5, 9, 14, 15, 24–26, 28, 29). The editing activity results in the insertion of a nontemplated G nucleotide at a specific site during transcription of the P gene which results in a frameshift and access to a different coding region. For measles and Sendai viruses, the P gene mRNA codes for the P protein, while the edited mRNA encodes the cysteine-rich V protein. In the other paramyxoviruses, simian virus 5, mumps virus, and the parainfluenza viruses, the P gene mRNA codes for the V protein and the edited version of the mRNA is translated into the P protein. Cattaneo et al. (5) have deduced a consensus editing sequence: 3′ UURRR CCC GUXRCR 5′, in which R is any purine and X is any nucleotide, based on the various viral P gene sequences, some of which were subsequently shown to be edited in vivo. It is postulated that the G insertion(s) occurs via a stuttering mechanism by the viral polymerase at the site of at least three consecutive C nucleotides in the genomic sequence (29).

To address the current questions in measles virus transcription, such as initiation and RNA editing, it was important to develop a system for transcription of purified virus. Seifried et al. (21) initially demonstrated RNA polymerase activity associated with purified measles virus. More recently, Ray and Fujinami (17) described in vitro transcription of isolated measles virus intracellular nucleocapsids; and we reported in vitro transcription with cytoplasmic extracts of measles virus-infected cells (12). We report here a transcription system with detergent-disrupted purified measles virus which we used to demonstrate the synthesis of both viral mRNAs and free leader RNA in vitro, suggesting that the mechanism of viral transcription is analogous to that of other negative-strand RNA viruses. Furthermore, we showed that measles virus P mRNA editing occurs during in vitro transcription with purified virus.

MATERIALS AND METHODS

Cells and virus. Early-passage (one to six) plaque-purified measles virus (Edmonston strain; American Type Culture Collection), was grown in monolayer cultures of Vero cells

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at a multiplicity of infection of 0.5 PFU per cell at 32.5°C (20, 27) for 3 or 4 days in Eagle's minimal essential medium containing 5% fetal calf serum and 0.2% bovine serum albumin (to a final concentration of 4 mg of protein per ml) (as discussed by Scott and Choppin [20]). The infected cells were scraped into the medium and pelleted by low-speed centrifugation. The cell-associated virus in the cell pellet was resuspended in phosphate-buffered saline, frozen at −70°C, thawed, and sonicated. The virus was titered on Vero cells and stored at −70°C. Measles virus released into the supernatant of the infected cells was pelleted through a 25% glycerol pad in HNE (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid] [pH 7.4], 1 mM EDTA, 100 mM NaCl) for 4 h at 4°C at 25,000 rpm in a Beckman SW28 rotor. The pelleted virus was purified by banding on 7 to 60% (wt/wt) sucrose gradients in HNE for 16 h at 4°C at 24,000 rpm in a Beckman SW41 rotor. The virus was pelleted, resuspended in buffer (1 mM Tris-HCl [pH 7.5], 1 mM EDTA) plus 10% dimethyl sulfoxide, and stored at −70°C. The yield was approximately 5.0 mg of purified measles virus from 1.6×10^9 Vero cells.

In vitro transcription. Purified measles virus was incubated at 30°C for 3 h in a reaction mixture (30 μg of purified virus per 100 μl) containing 0.1 M HEPES (pH 8.0), 0.05 M NH_4Cl, 7 mM KCl, 4.5 mM Mg acetate, 1 mM dithiothreitol, 1 mM spermidine, 2 μg of dactinomycin per ml, 10% glycerol, 0.05% Triton X-100, 1 unit of RNasin (Promega Biotech) per μl, 2 mM ATP, 1 mM each GTP and CTP, and 20 μM UTP plus [α^{32}P]UTP (300 μCi/ml) or 1 mM UTP for the synthesis of labeled or unlabeled RNA, respectively. Control reactions with no RNA synthesis lacked all nucleoside triphosphates (NTP) with the addition of 20 mM EDTA. The soluble protein fraction from uninfected A549 cells (USP) was prepared as described previously (2), and USP from 10^7 uninfected A549 cells was added for each 100 μl of reaction mixture as indicated in the text. SP6-directed synthesis of the P mRNA from the measles virus P gene cloned downstream of the SP6 promoter in the SP65 vector (a generous gift from W. Bellini, Centers for Disease Control, Atlanta, Ga.) was done according to the manufacturer's (Promega Biotec) instructions.

Isolation, purification, and analyses of RNA. A549 cells were infected with measles virus at a multiplicity of infection of 5 PFU per cell for 22 h at 37°C in the presence of 2 μg of dactinomycin per ml. After preparation of cytoplasmic cell extracts by lysolecithin treatment (16), the total infected cell RNA as well as RNA from in vitro reactions were purified by proteinase K digestion and phenol-chloroform extraction and analyzed by acid–urea–1.5% agarose gel electrophoresis.

The dot blot on Hybond N nitrocellulose (Amersham) was prepared with plasmid pBR322 DNAs containing the measles virus clones CL-15 (N), 5H5 (P), BA7 (M), IG-4 (F), and 11B-10 (H) (generously provided by W. Bellini) (18) or the Sendai virus P gene clone p92 (a gift from David Kingsbury, St. Jude Childrens' Hospital, Memphis, Tenn.). The DNA was blotted as described by Ray and Fujinami (17) with 0.5 μg of each viral sequence and UV irradiated to fix the DNA. The blot was then prehybridized and hybridized with in vitro ^{32}P-labeled RNA at 42°C in 50% deionized formamide–50 mM phosphate buffer (pH 6.4)–5× Denhardt's solution–0.1% sodium dodecyl sulfate (SDS)–100 μg of carrier DNA per ml for 4 and 16 h, respectively. The blot was then washed twice with 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate)–0.5% SDS for 5 min per wash at room temperature and then washed twice at 50°C in 0.1× SSC–0.1% SDS for 15 min per wash and exposed to Kodak XAR film.

To identify measles virus leader RNA, total unlabeled RNA from infected cells or from in vitro reactions was separated on an 8% polyacrylamide–8 M urea gel and electroblotted onto Hybond N nitrocellulose at 40 V for 24 h at 4°C in 10 mM Tris-HCl (pH 7.8)–5 mM Na acetate–0.5 mM EDTA. The blot was UV irradiated to fix the RNA and prehybridized and hybridized for 4 and 20 h, respectively, in 50% deionized formamide–5× Denhardt's solution–0.1% SDS–5× SSC–100 μg of salmon sperm DNA per ml at 30°C. The hybridization probe, an oligodeoxynucleotide having the sequence 5' AAGTG CACTA GAAGA TGATC ATTGA TTGAA CTATC CTTAC CCAAC TTTGT TTGGT 3', representing the 3' 55 bases of the measles virus genome RNA, the complement of leader RNA, was end labeled by polynucleotide kinase with [γ-^{32}P]ATP (Amersham). The radiolabeled probe was purified and added during hybridization. The blot was then washed at room temperature, first with 1× SSC–0.5% SDS and then with 0.1× SSC–0.1% SDS, twice each for 5 min, and then exposed to Kodak XAR film.

To test for RNA editing, the cytoplasmic extracts from measles virus-infected A549 cells, the in vitro transcription reaction (500 μl), and the SP6 transcription product of the measles virus P cDNA were treated with Nonidet P-40 (1%) and sodium deoxycholate (0.1%) and fractionated by centrifugation through 5.7 M CsCl for 16 h at 4°C at 36,000 rpm in a Beckman SW55 rotor. Under these conditions, the mRNA pelleted and was separated from any viral nucleocapsids which banded on the CsCl. The mRNA was resuspended in 10 mM Tris-HCl (pH 7.5)–1 mM EDTA and ethanol precipitated. A synthetic oligodeoxynucleotide primer (0.5 μg) 30 bases long, 5' ACGCG ATCTC CGTTC CAAAT GAGGC TAATC 3', complementary to the P mRNA sequence downstream of the editing site was hybridized to the viral mRNA at 30°C for 16 h in 80% deionized formamide–0.4 M NaCl–40 mM PIPES [piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)] (pH 6.4)–1 mM EDTA. The hybridized complex was ethanol precipitated and resuspended for the primer extension reaction (27 μl) containing 50 μM dCTP, 50 μCi of [α-^{32}P]dCTP, 0.5 mM each dGTP, TTP, and dideoxy ATP, 0.1 M Tris-HCl (pH 8.3), 10 mM $MgCl_2$, 0.14 M KCl, 40 U of avian myeloblastosis virus reverse transcriptase (Life Sciences), and 2 U of RNasin and incubated at 42°C for 90 min. The primer extension products were analyzed by electrophoresis on a 15% polyacrylamide–7 M urea sequencing gel, which was exposed to Kodak film.

The formation of functional edited RNA was also assayed by translating the in vivo- and in vitro-transcribed measles virus RNAs in rabbit reticulocyte lysates in vitro (Promega Biotec) in the presence of either [^{35}S]methionine or [^{35}S]cysteine (Amersham) at 150 μCi/25-μl reaction for 2 h at 30°C. The measles virus translation products were immunoprecipitated with human subacute sclerosing panencephalitis serum (2 μl of no. 267G from P. Dowling, New Jersey Medical School, Piscataway, N.J.), analyzed by electrophoresis on a 10% polyacrylamide–SDS gel, processed for fluorography, dried, and exposed to Kodak XAR film.

RESULTS

Transcription by purified measles virus. Measles virus released into the supernatant of infected Vero cells was purified by sucrose gradient centrifugation. Detergent-disrupted virus was transcribed in the presence of [α-^{32}P]UTP in reaction conditions that we previously showed were optimal for measles virus RNA synthesis in infected cell extracts (12). Purified measles virus alone appeared to synthesize at least the N, P/C, and M mRNAs of the correct

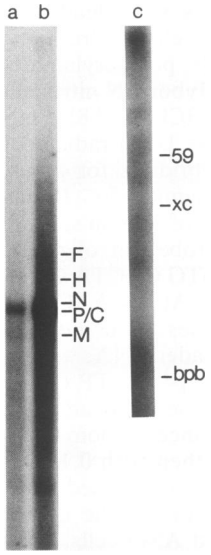

FIG. 1. In vitro transcription of purified measles virus. Sucrose gradient-purified measles virus (30 μg) was detergent disrupted and incubated in a reaction mixture (see Materials and Methods) in the presence (lane b) or absence (lanes a and c) of the soluble protein fraction (USP) from uninfected A549 cells. The labeled RNAs were purified and analyzed by electrophoresis on a 1.5% agarose–acid–urea gel (lanes a and b) or a 12% polyacrylamide–urea gel (lane c). The positions of the individual viral mRNAs were identified as indicated by Northern (RNA) blot analysis of RNA isolated from measles virus-infected A549 cells with measles virus-specific clones on this gel system. The N and P/C mRNAs comigrate. The positions of an RNA marker (59 bases) and xylene cyanol (xc) and bromphenol blue (bpb) are indicated.

lengths (Fig. 1, lane a). We have previously shown that both in vitro transcription of purified Sendai virus and measles virus RNA synthesis in infected cell extracts were enhanced by the addition of the soluble protein fraction of uninfected cells (USP) (12, 13). Similarly, overall in vitro transcription by purified measles virus was stimulated sevenfold by USP (Fig. 1, lane b). As mentioned previously, measles virus leader RNA had not been detected in infected cells (3, 6). It is possible that measles virus leader RNA either is not synthesized or is very unstable in infected cells. Testing for synthesis of leader RNA from purified measles virus should not have the latter problem, and in fact, analysis of the small RNA transcription products showed the synthesis of several small RNA species (Fig. 1, lane c). One species appeared to be about 55 nucleotides long and would, therefore, be a candidate for the measles virus leader RNA (see below).

To confirm the identity of the product RNAs, we hybridized ^{32}P-labeled RNAs synthesized by purified virus in the presence (Fig. 2) and absence (data not shown) of USP to a dot blot containing clones of various measles virus genes. The data show that purified measles virus synthesized authentic viral N, P, M, F, and H mRNAs (Fig. 2). The relative abundance of the mRNAs showed polarity depending on the distance of the gene from the 3′ end (Table 1). No clone was available to quantitate L mRNA synthesis. There was no apparent difference in the relative abundances of the viral mRNAs synthesized in the absence or presence of USP (Table 1), although in the latter case there was an overall stimulation of all the RNAs (Fig. 1, lanes a and b).

Identification of measles virus leader RNA synthesized in vitro. We demonstrated above the synthesis of several small

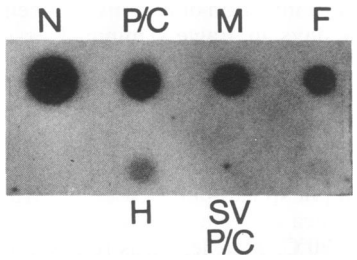

FIG. 2. Identification of measles virus mRNAs synthesized by purified virus. A dot blot with specific measles virus clones (see Materials and Methods) was hybridized to total measles virus RNA synthesized in vitro with purified measles virus (60 μg) in the presence of [α-^{32}P]UTP and USP as described in Materials and Methods. The Sendai virus P gene clone (SV P/C) was included in the blot as a negative control.

radiolabeled RNAs by purified measles virus in vitro (Fig. 1, lane c) but needed to determine which, if any, of these were leader specific. Unlabeled RNAs synthesized by purified measles virus in the presence or absence of USP were separated by electrophoresis and electroblotted onto Hybond N nitrocellulose. Hybridization of the blot with a ^{32}P-end-labeled probe corresponding to the exact 3′ end of measles virus genome RNA, the leader gene, showed that measles virus synthesized multiple leader-specific RNAs ranging from about 39 to 55 nucleotides whose synthesis was stimulated by USP (Fig. 3, lanes d and c, respectively). In addition to a major band at 55 nucleotides (species 1), the presumed leader RNA, an abundant leader-specific RNA of about 42 nucleotides (species 2), was also synthesized. This and the other smaller RNAs were probably products of early termination by the viral polymerase. The leader-specific RNAs were de novo transcription products (Fig. 3, lane f), since no leader-specific RNA appeared in a sample in the absence of RNA synthesis (Fig. 3, lane e). Thus, leader RNAs were not packaged in measles virus particles.

Previous studies had been unable to demonstrate any leader RNA in a variety of virus-infected cells (3, 6), yet purified virus does synthesize leader RNA. This product must be degraded in the infected cells. We tested whether leader RNA was stable in the cell lines used for infections in our laboratory. Unlabeled RNA was isolated from measles virus-infected Vero and A549 cells and analyzed on the same blot as above. It is clear that measles virus leader RNA could be detected in infected A549 cells but not in infected Vero

TABLE 1. Abundance of mRNAs transcribed by measles virus in vitro

Measles virus RNA	Relative abundance of mRNAs (%)[a]	
	Alone	+USP
N	70	72
P/C	18	17
M	9	6
F	3	4
H	0.1	1

[a] Purified measles virus (60 μg) was detergent disrupted and transcribed in the presence of [α-^{32}P]UTP in the absence or presence of USP as described in Materials and Methods. The RNAs were purified and hybridized to dot blots of measles virus clones (Fig. 2) and quantitated with an AMBIS beta-scanner. The relative abundance of each of the RNAs was determined as a percentage of the total counts in all five mRNAs.

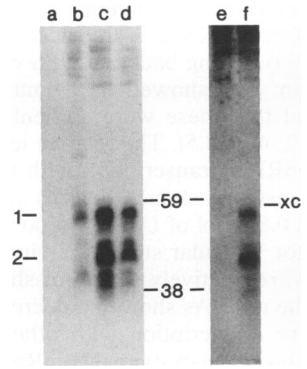

FIG. 3. Identification of measles virus leader RNA synthesized in vivo and in vitro. Unlabeled total measles virus RNA was isolated from 4×10^7 Vero (lane a) or A549 (lane b) infected cells as described in Materials and Methods. In vitro-synthesized unlabeled RNAs were isolated from transcription reactions containing purified measles virus (60 μg) in the presence (lane c) or absence (lanes d to f) of USP or from a mock reaction with no RNA synthesis (−NTP + 20 mM EDTA) (lane e). The RNA was separated by electrophoresis on an 8% acrylamide–urea gel, electroblotted onto Hybond N nitrocellulose, and hybridized to a ^{32}P-end-labeled leader probe (see Materials and Methods). 1 and 2 are leader-specific RNAs of approximately 55 and 42 bases, respectively. The positions of marker RNAs of 59 and 38 bases and xylene cyanol (xc) are indicated.

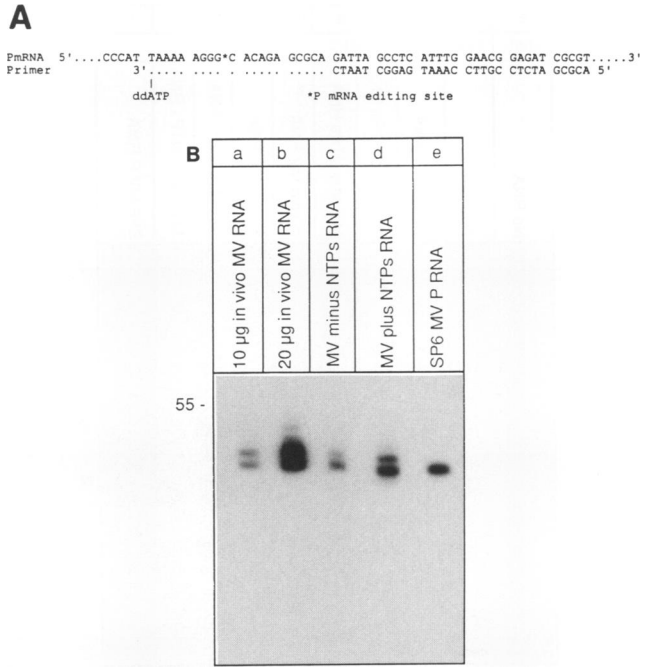

FIG. 4. Measles virus (MV) P mRNA editing in vivo and in vitro. (A) Synthetic oligonucleotide primer 30 bases long is shown hybridized downstream of the editing site on the P mRNA. The expected primer extension product synthesized by reverse transcriptase in the presence of dCTP, dGTP, TTP, and ddATP is indicated by the dots, and its size depends on the number of G residues present in the mRNA at the editing site. (B) Oligonucleotide primer was hybridized to 10 or 20 μg of total RNA from measles virus-infected A549 cells (lanes a and b); control RNA from purified virus (300 μg) plus USP in the absence of RNA synthesis (−NTP plus 20 mM EDTA) (lane c); RNA transcribed (plus NTP) by detergent-disrupted purified measles virus (300 μg/0.5-ml reaction plus NTP) (lane d); and P mRNA transcribed from an SP65 P gene expression plasmid (lane e) as described in Materials and Methods. The primer was extended with avian myeloblastosis virus reverse transcriptase in the presence of [^{32}P]dCTP, and the products were analyzed by polyacrylamide-urea gel electrophoresis. The position of a marker DNA of 55 bases is indicated.

cells (Fig. 3, lanes b and a, respectively). Although measles virus grows relatively well in Vero cells, the leader RNA was probably degraded in these cells as observed by others. In contrast, infected A549 cells seemed to accumulate both leader RNA (Fig. 3) and replicative intermediates (12).

P mRNA editing in vitro. P mRNA editing has been shown to occur in cells infected with a number of paramyxoviruses (5, 9, 14, 15, 24–26, 28, 29). In addition, Vidal et al. (28, 29) showed that purified Sendai virus edited P mRNA in vitro, suggesting that this activity was virus encoded. To test whether P mRNA editing also occurred during in vitro transcription with purified measles virus, we wanted to utilize a simpler assay than cloning and sequencing the product RNAs as had been done previously (5). As shown in Fig. 4A, the P mRNA can be hybridized to a synthetic oligodeoxynucleotide, 30 bases long, that is complementary to the mRNA downstream of the site of editing (5). Primer extension of the hybrid with reverse transcriptase in the presence of [α-^{32}P]dCTP, dGTP, TTP, and ddATP would permit the synthesis of a product that would terminate at the first U residue (T in the DNA form) after the editing site. The length of the product will thus depend on the number of nontemplated Gs, if any, that were inserted at the editing site. As a control for the specificity of the reaction, primer extension from the P mRNA transcribed in vitro from a cloned measles virus unedited P gene resulted in the synthesis solely of a 50-mer as predicted (Fig. 4B, lane e).

When the primer extension assay was used with total mRNA isolated from measles virus-infected A549 cells, there were two major products found in approximately equal amounts: one (50-mer) from the unedited P mRNA and one (51-mer) with one additional G from the V mRNA (Fig. 4B, lane a). There were also some longer products present in low abundance, representing the insertion of two to eight Gs that were better visualized when twice as much mRNA was analyzed in the primer extension reaction (Fig. 4B, lane b). In addition, the amount of the extension product was proportional to the amount of added template. These data were consistent with the approximately equal amounts of the P and V mRNAs found in measles virus-infected cells as determined by a different methodology (5). An identical analysis was performed with CsCl-pelleted, unlabeled RNA transcribed in vitro from purified measles virus. Some viral mRNAs were apparently packaged into virions since the assay detected low levels of primer extension products in a control reaction in which no RNA synthesis occurred (Fig. 4B, lane c). There was, however, clearly a significant increase in the level of the primer extension products consistent with RNA transcription from virus (Fig. 4B, lane d) compared with the background level (lane c). In the virus transcription products, both the unedited P mRNA and the edited V mRNA (the 50-mer and 51-mer, respectively) were detected (Fig. 4B, lane d); however, there appeared to be a lower level of editing (about 30% of the total). A very low level of RNAs having more than one added G was also detected.

Cattaneo et al. (5) showed by in vitro translation of the mRNAs from measles virus-infected cells and cloned genes that the P protein was translated from the unedited mRNA and the V protein was translated from the edited mRNA. Translational analysis of the RNA synthesized by purified virus was also used to confirm the formation of V mRNA in

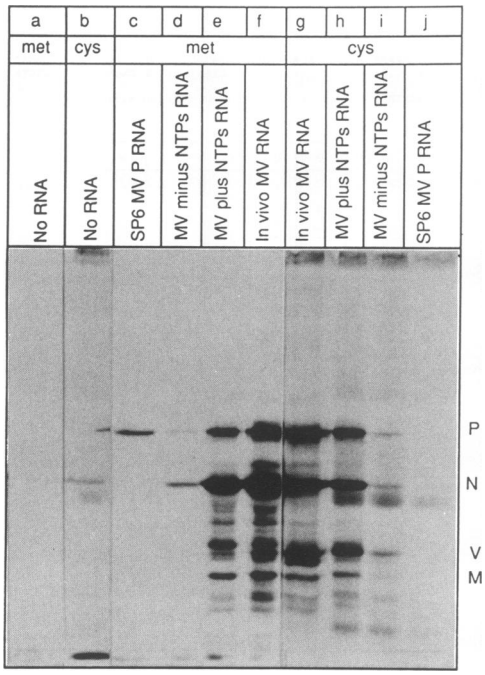

FIG. 5. In vitro translation of measles virus (MV) in vivo and in vitro RNAs. No RNA (lanes a and b) or 20 μg of measles virus RNA isolated from infected A549 cells (lanes f and g), the P mRNA transcribed from the cloned P gene (lanes c and j), and the total RNA from purified virus (300 μg/0.5-ml reaction) in the presence of USP plus NTP (lanes e and h) or minus NTP (no RNA synthesis) (lanes d and i) were translated in a rabbit reticulocyte lysate labeled with [^{35}S]methionine (lanes a and c to f) or [^{35}S]cysteine (lanes b and g to j). One-third of the [^{35}S]methionine or all of the [^{35}S]cysteine translation products were immunoprecipitated with anti-SSPE serum and analyzed by polyacrylamide-SDS gel electrophoresis. Lanes b and g to j were exposed ten times longer than lanes a and c to f.

vitro. Translation in the absence of RNA showed little or no protein synthesis, as expected (Fig. 5, lanes a and b). Translation of in vivo measles virus mRNA showed that by cysteine labeling, the level of V protein was enhanced relative to that of P protein, compared with methionine labeling of the proteins (Fig. 5, lanes f and g) as reported previously (5). For these experiments, the translation products were immunoprecipitated with a human anti-SSPE serum which was found to recognize the V protein as well as the other viral proteins. The low level of mRNA packaged in virions gave poor translation (Fig. 5, lanes d and i), while the RNA synthesized by virus in vitro gave translation of a differentially [^{35}S]cysteine-labeled V protein as well as the synthesis of full-length proteins corresponding to P, N, and M (Fig. 5, lanes e and h). Thus, translation analysis showed that functional mRNAs for the viral proteins, including V, were synthesized in vitro by purified virus.

DISCUSSION

In vitro transcription requires significant amounts of purified virus. For measles virus, this has been difficult, since the virus does not grow to high titers and is relatively unstable. We have been able to grow measles virus successfully in monolayer cultures of Vero cells by growing the virus at 32.5°C and by adding excess protein to 4 mg/ml in the infection medium to help stabilize the virus (20). The virus was purified by conventional banding techniques. Although small amounts of viral mRNAs were packaged within purified virus (Fig. 4 and 5), giving background levels in the assays, RNA synthesis in vitro showed the stimulation of mRNA. We demonstrated that these were authentic and functional mRNAs (Fig. 1, 2, 4, and 5). The relative level of nucleocapsid protein (N) mRNA transcription with measles virus fell between that of vesicular stomatitis virus and Sendai viruses with 64, 3.2, and 0.8 pmol of UTP incorporated into N RNA per μg of virus for vesicular stomatitis virus, measles virus, and Sendai virus, respectively (data not shown). Moreover, the amounts of the mRNAs showed a decrease in expression similar to in vitro transcription with other negative-strand viruses, with the promoter-proximal N mRNA being the most abundant, followed by the P/C, M, F, and H mRNAs in decreasing abundances. These data agree overall with those of Ray and Fujinami (17), who quantitated the mRNAs that were synthesized during in vitro transcription with measles virus nucleocapsids isolated from infected cells. As percentages of the total, the N and P/C mRNAs in both systems were about 70 and 20%, respectively; however, the relative amounts of the distal mRNAs differed. The amounts of the M, F, and H mRNAs synthesized by purified virus decreased (M, 9%; F, 3%; and H, 0.1%) (Table 1), whereas these mRNAs increased in abundance (M, 1.5%; F, 2.4%; and H, 3%) when transcribed from purified intracellular nucleocapsids (17). The reason for this difference in the two systems is unknown. We did not determine the level of L mRNA synthesized, but Ray and Fujinami (17) showed that in their system the L mRNA composed 1% of the total. Neither of the in vitro systems synthesized the distal mRNAs with the same efficiency found in the infected cells, in which M, F, and H syntheses represent 35, 28, and 18% of the total RNA, respectively (30).

Castaneda and Wong (3) and Crowley et al. (6) had been unable to demonstrate the presence of free measles virus leader RNA in infected cells, and it was suggested that either this virus has a transcription strategy different from that of other negative-strand RNA viruses or the leader RNA was unstable in infected cells. Transcription with purified virus allowed us to demonstrate that the latter was correct. In vitro, purified measles virus synthesized a leader-specific RNA of about 55 nucleotides (Fig. 3). Transcription did, therefore, initiate directly at the 3′ end of the virion RNA, releasing free leader RNA. Furthermore, we were able to detect viral leader RNA in infected A549 cells but not in infected Vero cells (Fig. 3). Hence, the detection of free leader in measles virus-infected cells was dependent on the cell type utilized in the infection. By the same methodology, we were unable to detect virus leader RNA packaged in the purified measles virus (Fig. 3), unlike what has been reported for purified Sendai virus (10).

Viral RNA analysis showed a stimulation of measles virus leader RNA synthesis, as well as mRNA synthesis, when USP from uninfected A549 cells was added to the transcription reaction (Fig. 1 and 3). We have previously reported an increase in measles virus RNA synthesis from intracellular nucleocapsids with the addition of A549 USP and, furthermore, that tubulin is one of possibly several components involved in the stimulation (12). The stimulatory effect of USP, however, was cell type dependent, since USP from Vero cells inhibited RNA synthesis by purified measles virus (data not shown). This result was somewhat unexpected, since Vero cells are normally used to grow measles virus, and may reflect an increased RNase activity induced by the preparation of extracts from these cells. The absence of

leader RNA in Vero cells as discussed above perhaps reflects a low level of endogenous RNase in intact Vero cells as well.

Measles virus and Sendai virus have been previously shown to synthesize P mRNAs that have one G nucleotide inserted at a specific site, termed RNA editing, which resulted in an altered P mRNA that encodes a cysteine-rich V protein (5, 28), whose function is currently unknown. As with Sendai virus (29), we showed here that purified measles virus could also edit the P mRNA in vitro (Fig. 4), suggesting that this activity is virus encoded. The proportion of unedited to edited RNA appeared to differ somewhat from viral RNA isolated from infected cells. The in vivo-edited V mRNA constituted between 45 and 50% of the P gene transcription products, similar to that reported by Cattaneo et al. (5), by cloning and sequencing of cDNAs from intracellular viral mRNAs. In contrast, purified measles virus incorporated nontemplated G nucleotides to a lesser degree in vitro (30%), suggesting that the host cell plays some role in the editing reaction during measles virus infection. A similar primer extension analysis of RNA from purified intracellular nucleocapsids showed that no detectable editing occurred during positive-strand genome RNA replication (13a). Encapsidation during replicative RNA synthesis apparently suppresses the editing reaction. Sendai virus edited the P mRNA in vivo (38%), while in vitro only 25% was edited, showing a decrease in vitro (28) similar to that observed here. The availability of a transcription system from purified measles virus should now facilitate further study of the mechanism of RNA editing.

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