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Leader Sequence Distinguishes between Translatable and Encapsidated Measles Virus RNAs

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The 3′-terminal 55 nucleotides of the negative-strand measles virus RNA genome called the leader sequence is not transcribed into a detectable distinct RNA product. Most of the monocistronic N and bicistronic N-P RNAs lack the leader sequence. However, a subpopulation of the N and N-P RNAs and all of the antigenomes possess this leader. Here, we show that leader-containing subgenomic RNAs are functionally distinct from their leaderless counterparts. In measles virus-infected cells, leaderless monocistronic N and bicistronic N-P RNAs were associated with polysomes. By contrast, leader-containing N and N-P RNAs were found exclusively in nonpolysomal ribonucleoprotein complexes that were resistant to RNase and had a buoyant density of 1.30 g/ml, the same as that of antigenomic ribonucleoprotein complexes. Both antigenomic and subgenomic ribonucleoprotein complexes were specifically immunoprecipitated by antiserum against the N protein, and leaderless RNAs were not found in these complexes. These findings suggest that measles virus distinguishes RNAs destined for encapsidation or translation by the presence or absence of a leader sequence.

Measles virus, a member of the morbillivirus subgroup of the paramyxovirus family, has a nonsegmented minus-sense RNA genome that is tightly encapsidated by nucleoprotein (N) into a nucleocapsid structure (19). In addition to N, the viral genome encodes for phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin (H), a presumed polymerase (L), and two nonstructural proteins, C and V, of unknown functions (1, 4–6, 7, 11, 25, 27). Protein C is encoded by an alternative reading frame in the P cistron, and protein V is made from edited P transcripts (5, 11). The cistrons that encode these proteins are arranged in the order 3′-N-P/V/C-M-F-H-L-5′ (15, 26, 37). Preceding the N gene at the 3′ genomic terminus is a sequence 55 nucleotides (nt) in length that is termed the leader (6, 14).

The genome of measles virus serves as a template for synthesis of both monocistronic- and polycistronic-length mRNAs as well as full-length plus-sense RNAs called antigenomes, which are presumably used for minus-sense genome replication (19). We recently reported that measles virus synthesized two distinct types of monocistronic and bicistronic N-containing RNAs. The majority of these species had 5′ termini that mapped to the beginning of the N cistron at nt 56, but a small portion of polyadenylated monocistronic N and bicistronic N-P RNAs contained a leader sequence at their 5′ ends, as was found for all antigenomic RNA. The leader sequence was not transcribed into a detectable independent 55-nt RNA species (10). These findings raised interesting questions about a possible function for the leader sequence in certain subgenomic measles virus RNAs.

In this study, we examined the association of measles virus RNAs with ribosomes and viral proteins in infected cells. The results showed that leaderless and leader-containing measles virus RNA species were functionally distinct. Leaderless RNAs of monocistronic and bicistronic lengths were both associated with polyribosomes, whereas leader-containing monocistronic and bicistronic RNAs were present in nonpolysomal ribonucleoprotein (RNP) complexes. These latter RNP complexes were immunoprecipitable with antiserum specific for N protein and exhibited a buoyant density the same as that of antigenomic RNP complexes. These results suggest that the plus-sense measles virus leader sequence serves as an encapsidation signal and that the absence or presence of this sequence distinguishes between translatable and encapsidated measles virus RNAs in vivo.

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MATERIALS AND METHODS

Cells and virus. African green monkey kidney (CV-1) cells were maintained at 37°C in Eagle minimal essential medium containing 10% fetal bovine serum. Cells infected with the Edmonston strain of measles virus were maintained in the same medium containing 2% fetal bovine serum.

Analysis of virus-specific RNP complexes by sucrose sedimentation gradients. When 100% of virus-infected CV-1 cells showed cytopathic effects at 37 h postinfection, cells were washed twice with cold phosphate-buffered saline (0.137 M NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4) and suspended in a total volume of 1 ml in RSB (10 mM Tris chloride [pH 7.4], 10 mM NaCl, 1.5 mM $MgCl_2$) containing 50 μg of heparin (Sigma Chemical Co., St. Louis, Mo.) per ml, 50 μg of cycloheximide (Sigma) per ml, and 1% Triton X-100 (Sigma). Cells were lysed with 10 to 12 strokes in a Dounce homogenizer, and nuclei were removed by centrifugation at 1,000 × *g* for 5 min at 4°C (33, 36). Equal portions of the lysate without or with EDTA treatment (10 mM, final concentration) were layered onto 10 to 30% (wt/vol) continuous sucrose gradients made with 25 mM Tris chloride (pH 7.5)–25 mM NaCl–5 mM $MgCl_2$–0.5 μg of heparin per ml and centrifuged at 190,000 × *g* for 100 min at 4°C in an SW41.Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.). Equal 0.5-ml fractions were collected, and RNA was purified as described below.

Analysis of nucleocapsid RNA by CsCl equilibrium density gradients. When 60% of virus-infected CV-1 cells showed cytopathic effects at 30 h postinfection, cells were washed twice with cold phosphate-buffered saline, lysed in 1 ml of 20 mM *N*-2-hydroxyethylpiperazine-*N*-2′-ethanesulfonic acid (HEPES; pH 7.4)–10 mM KCl–1.5 mM $MgCl_2$–1% Triton X-100 with 10 to 12 strokes in a Dounce homogenizer, and

centrifuged at 1,000 × *g* for 5 min at 4°C. Equal portions of the supernatant without or with EDTA treatment (5 mM, final concentration) were loaded onto 20 to 40% (wt/wt) continuous CsCl gradients made with 20 mM HEPES (pH 7.4)–25 mM NaCl–5 mM $MgCl_2$ and centrifuged at 111,000 × *g* for 52 h at 10°C in a Beckman SW41.Ti rotor. Equal 0.75-ml fractions were collected, and RNA was purified as described below. The pelleted material was suspended in a small volume of 4 M guanidine thiocyanate, and RNA was purified as described above for gradient fractions.

Immunoprecipitation of RNPs. When 90% of virus-infected CV-1 cells showed cytopathic effects at 35 h postinfection, cell homogenates were prepared as described above for virus-specific RNP complexes and incubated for 60 min at 4°C with antiserum against the N or H protein of measles virus. Some samples were treated with 10 mM EDTA at 4°C or 10 μg of RNase A (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml for 20 min at room temperature before immunoprecipitation. Immune complexes were adsorbed with 5 mg of protein A-Sepharose (Pharmacia, Inc., Piscataway, N.J.) in 50 μl of RSB for 15 min at 4°C and extensively washed with RSB, and RNA was purified as described below.

Antisera. To prepare a polyclonal antiserum against the measles virus N protein, the *Eco*RV fragment of pcD-N7, a previously described full-length N cDNA, was placed downstream of the inducible *trpE* promoter in the pATH vector (24, 34). When transformed into *Escherichia coli* HB101 and induced by tryptophan starvation and addition of indoleacrylic acid, this construct produced a fusion protein consisting of TrpE and the amino half of the N protein. This protein was purified from the insoluble protein fraction by electrophoresis in a sodium dodecyl sulfate–10% polyacrylamide gel, electroeluted, and used to immunize a female rabbit as previously described (29). Preparation of mouse monoclonal antiserum against the measles virus H protein has been described elsewhere (2).

RNA isolation and analyses. Cells, cell lysates, or gradient fractions were added to 4 M guanidine thiocyanate and centrifuged through a CsCl cushion (1.72 g/ml) at 110,000 × *g* for 16 to 20 h at 20°C in a Beckman SW50.1 rotor (12, 16). RNA pellets were dried, dissolved in 300 μl of TSE (10 mM Tris chloride [pH 7.5], 1 mM EDTA, 0.2% sodium dodecyl sulfate) and precipitated with 0.3 M sodium acetate and 3 volumes of ethanol at −70°C.

For Northern (RNA) blot analysis, RNA was electrophoresed in denaturing 1% agarose gels containing 2.2 M formaldehyde, transferred to nitrocellulose membranes, and hybridized with a ^{32}P-labeled minus-sense N-specific riboprobe transcribed in vitro by T7 RNA polymerase (Boehringer Mannheim) from the *Xho*I-*Xba*I fragment of pcD-N7 in a pGEM vector (Promega Biotec, Madison, Wis.) (22, 23).

For RNase protection assay, a ^{32}P-labeled 354-nt minus-sense leader-N riboprobe was transcribed in vitro from a linearized plasmid by T7 RNA polymerase, hybridized overnight with RNA samples at 55°C, RNase digested at 30°C, and analyzed on 8 M urea–6% polyacrylamide sequencing gels as previously described (10).

RESULTS

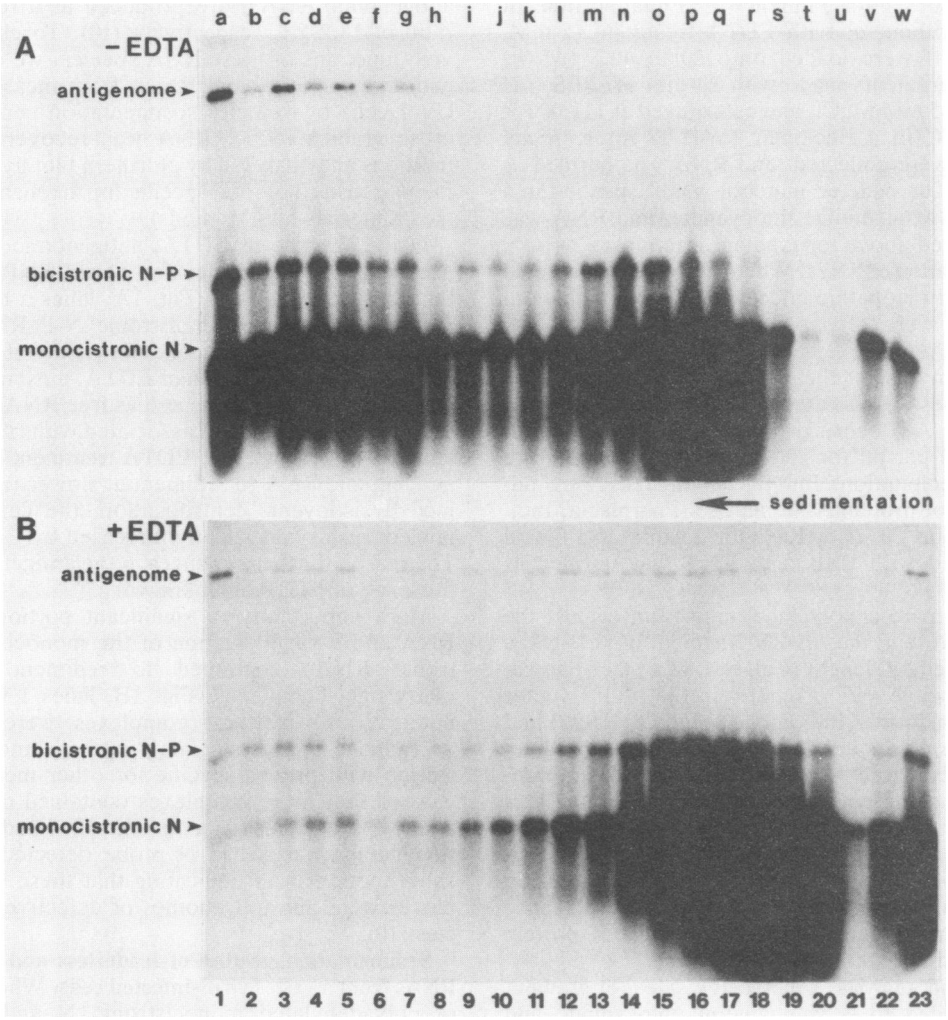
Sedimentation profile of monocistronic N, bicistronic N-P, and antigenomic RNA species from measles virus-infected cells. Measles virus synthesizes three different lengths of plus-sense RNAs that contain N sequences (10, 15, 26, 37). These species, called monocistronic N, bicistronic N-P, and antigenomic RNAs, are produced at a relatively constant ratio of 18:2.5:1, respectively (10). To characterize the in vivo functions of these RNA species, we examined polysomal and nonpolysomal RNAs from measles virus-infected CV-1 cells by velocity sedimentation centrifugation in sucrose gradients (33). RNA was recovered from gradient fractions and analyzed by Northern blot hybridization with a minus-sense riboprobe specific for the measles virus N gene (see Materials and Methods).

In the absence of EDTA, antigenomic RNA sedimented exclusively as high-molecular-weight RNP complexes to the bottom of the gradient (Fig. 1A, lanes a to g). In contrast, monocistronic N and bicistronic N-P RNAs appeared to sediment as monosomes and polysomes (Fig. 1A, lanes a to s; Fig. 1C). After addition of EDTA, most monocistronic and bicistronic RNAs sedimented as free RNAs, confirming that these RNA species were associated with ribosomes (Fig. 1B, lanes 12 to 21; Fig. 1C). EDTA treatment also caused some antigenomic RNA to sediment at slower rates. When examined by isopycnic centrifugation, the density of the viral nucleocapsids was slightly increased by EDTA (see Fig. 3). The reason for these changes in the antigenomic RNA in the presence of EDTA is unknown.

More importantly, a significant portion of antigenomic RNA and a small fraction of the monocistronic and bicistronic RNAs continued to sediment as high-molecular-weight complexes (Fig. 1B, lanes 1 to 5). The subgenomic EDTA-resistant complexes were unlikely to be degradative products of antigenomes, since similar hybridization with probes specific for other measles virus genes showed that these complexes contained only plus-sense N and N-P sequences (data not shown). In addition, hybridization with a plus-sense N probe detected no minus-sense subgenomic RNAs, indicating that these subgenomic complexes were not antigenomes of defective interfering particles (10).

Sedimentation profiles of leaderless and leader-containing RNAs from measles virus-infected cells. Whereas the majority of polyadenylated monocistronic N and bicistronic N-P RNAs synthesized by measles virus are leaderless, approximately 2% of these species and all the antigenomes have a 5′-terminal leader sequence (10). To determine whether the presence or absence of a leader sequence might correlate with the functional disparity observed for some of these subgenomic RNA species, we analyzed the same sucrose gradient fractions by an RNase protection assay, using a 354-nt minus-sense leader-N riboprobe that includes the first 179 nt of the measles virus 3′ genomic end (see Materials and Methods).

As previously described, leaderless N and N-P RNAs of measles virus protected a 123-nt fragment with this probe, whereas leader-containing N and N-P RNAs protected a 179-nt fragment (Fig. 2, MV lane; 10). In the absence of EDTA, a 123-nt fragment representing leaderless N RNA was found throughout the gradient in amounts approximately proportional to the relative abundance of monocistronic N and bicistronic N-P RNA species (compare Fig. 2A with Fig. 1A, lanes a to s). However, RNA in the fractions containing the higher-molecular-weight complexes protected an additional 179-nt fragment that represented leader-containing RNA (compare Fig. 2A with Fig. 1A, lanes a to g). Addition of EDTA separated leaderless and leader-containing RNAs into two distinct populations. Most of the RNAs released from polysomes by EDTA treatment lacked a leader sequence and protected only a 123-nt fragment (Fig. 2B, lanes 12 to 21). In contrast, essentially all of the RNAs



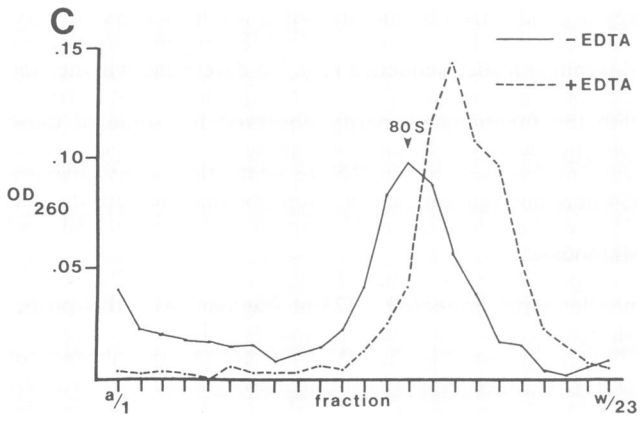


FIG. 1. Sedimentation centrifugation of plus-sense N-containing RNAs. Homogenates from measles virus-infected CV-1 cells were prepared without (A) or with (B) EDTA and centrifuged through continuous 10 to 30% sucrose sedimentation gradients. Collected fractions were added to 4 M guanide thiocyanate, and RNA was isolated by centrifugation through CsCl (see Materials and Methods). One-fourth of the resulting RNA was electrophoresed in denaturing 1% agarose gels and analyzed by Northern blot hybridization with a minus-sense N-specific riboprobe. (C) Graph depicting optical density at 260 nm of each fraction as determined by spectrophotometry.

that still sedimented as high-molecular-weight complexes in the presence of EDTA contained a leader sequence, as indicated by a 179-nt fragment (Fig. 2B, lanes 1 to 5). In this experiment and in that presented in Fig. 4 (see below), the minor bands above and below the 179-nt fragment resulted from nonspecific protection of the riboprobe by host cell RNA, since these bands were also observed with RNA from mock-infected cells (Fig. 2A and B, mock lanes) and were not seen when the probe alone was digested with RNase (data not shown).

Antigenomes and leader-containing subgenomic RNP complexes have the same buoyant density. To further characterize the high-molecular-weight complexes containing genomic- and subgenomic-length RNAs, lysates from measles virus-infected CV-1 cells were centrifuged to equilibrium in CsCl gradients to separate viral nucleocapsids from other cytoplasmic RNAs, including mRNAs (9; see Materials and Methods). RNA was isolated from the collected fractions or pelleted material and analyzed by Northern blot hybridization with a minus-sense N-specific riboprobe.

All antigenomic RNA banded at an average density of 1.30 g/ml, corresponding to the buoyant density of measles virus nucleocapsids (Fig. 3A, lanes g to i; Fig. 3C; 30, 31). Significantly, a small fraction of monocistronic N and bicistronic N-P RNAs banded at a density identical to that of

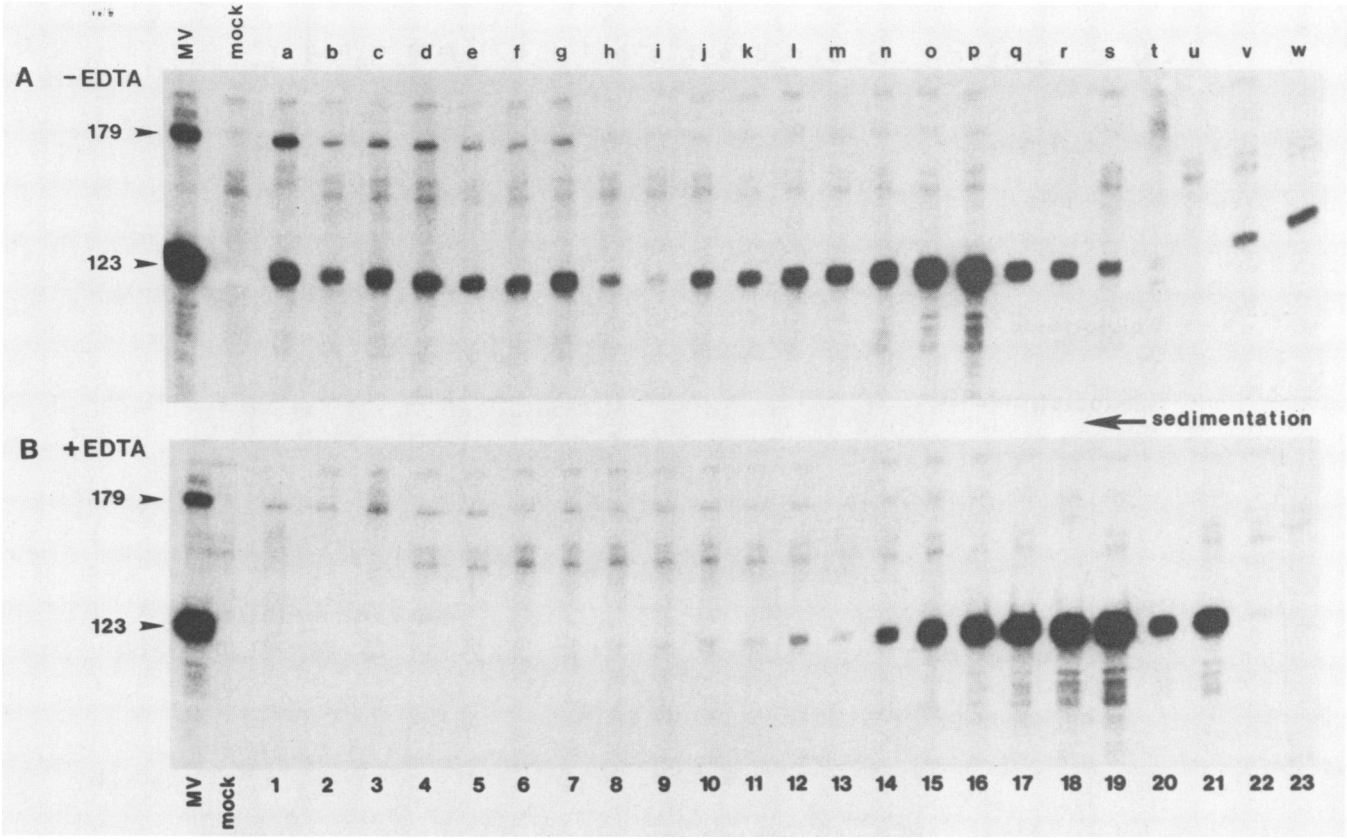


FIG. 2. Sedimentation centrifugation of leader-containing and leaderless RNAs. One-fourth of each RNA preparation recovered from the gradient fractions shown in Fig. 1 or 2.5 μg of total RNA from uninfected (mock) or measles virus-infected (MV) CV-1 cells was hybridized with an excess molar amount of minus-sense leader-N riboprobe at 55°C and digested with RNases A and T_1 at 30°C. Protected fragments were resolved in a 6% sequencing gel. The fractions are designated with the same letters or numbers as for Fig. 1.

antigenomes (Fig. 3A, lanes g to i). These subgenomic-length RNPs were insensitive to EDTA treatment, indicating these species were not polysome associated (Fig. 3B, lanes 7 to 9). However, in the presence of EDTA, these genomic- and subgenomic-length RNPs banded at a slightly higher density of 1.32 g/ml (compare Fig. 3B, lanes 7 to 9, and Fig. 3A, lanes g to i; Fig. 3C). This result suggested that EDTA might dissociate some protein or proteins from these RNP complexes, which might explain the lower recovery of fast-sedimenting complexes when lysates were treated with EDTA before centrifugation in sucrose gradients (Fig. 1B).

Regardless of the presence or absence of EDTA, the majority of monocistronic N and bicistronic N-P RNAs were pelleted (Fig. 3A, lane a; Fig. 3B, lane 1). These RNA species had a buoyant density of greater than 1.48 to 1.50 g/ml (Fig. 3C) and were therefore not extensively complexed with proteins. The bicistronic N-P RNA species was significantly degraded in this experiment but was clearly visible in another experiment using a shorter centrifugation time.

When the same CsCl gradient fractions were analyzed by the RNase protection assay with a minus-sense leader-N riboprobe, leader-containing and leaderless RNAs were again found in distinct populations. The banded RNP-associated RNA protected a 179-nt fragment corresponding to leader-containing RNAs (Fig. 4A, lanes g to i; 4B, lanes 7 to 9, arrowheads). However, RNA recovered from the pelleted material was leaderless and protected a 123-nt fragment (Fig. 4A, lane a; Fig. 4B, lane 1, arrowheads). As described for Fig. 2, nonspecific bands also present in RNA from mock-infected cells were due to aberrant protection by cellular RNAs (Fig. 4B, mock lane).

Together, the results described above demonstrate that the leaderless and leader-containing RNAs synthesized by measles virus are found in distinct RNP complexes in vivo. Leaderless RNAs of both monocistronic N and bicistronic N-P classes are associated with ribosomes. In contrast, RNAs with a leader sequence, including antigenomes and a subpopulation of monocistronic N and bicistronic N-P RNAs, form nonpolysomal RNP complexes.

N protein specifically encapsidates leader-containing RNAs. Genomic and antigenomic RNAs of paramyxoviruses are tightly encapsidated by nucleoprotein into nucleocapsids that are resistant to RNase digestion (19). To investigate whether leader-containing subgenomic RNPs were similarly associated with N protein, we used a polyclonal antiserum against the measles virus N protein to immunoprecipitate RNP complexes from measles virus-infected CV-1 cell lysates and examined the RNA present in these complexes (see Materials and Methods).

The anti-N serum immunoprecipitated not only antigenomes but also monocistronic N and bicistronic N-P RNAs (Fig. 5A, lanes d and e). Immunoprecipitation of these RNAs was specific, since these species remained in the supernatant if the cell lysate was centrifuged alone and were not adsorbed by protein A-Sepharose in the absence of antisera (Fig. 5A, lanes b, a, and c, respectively). The immunopre-

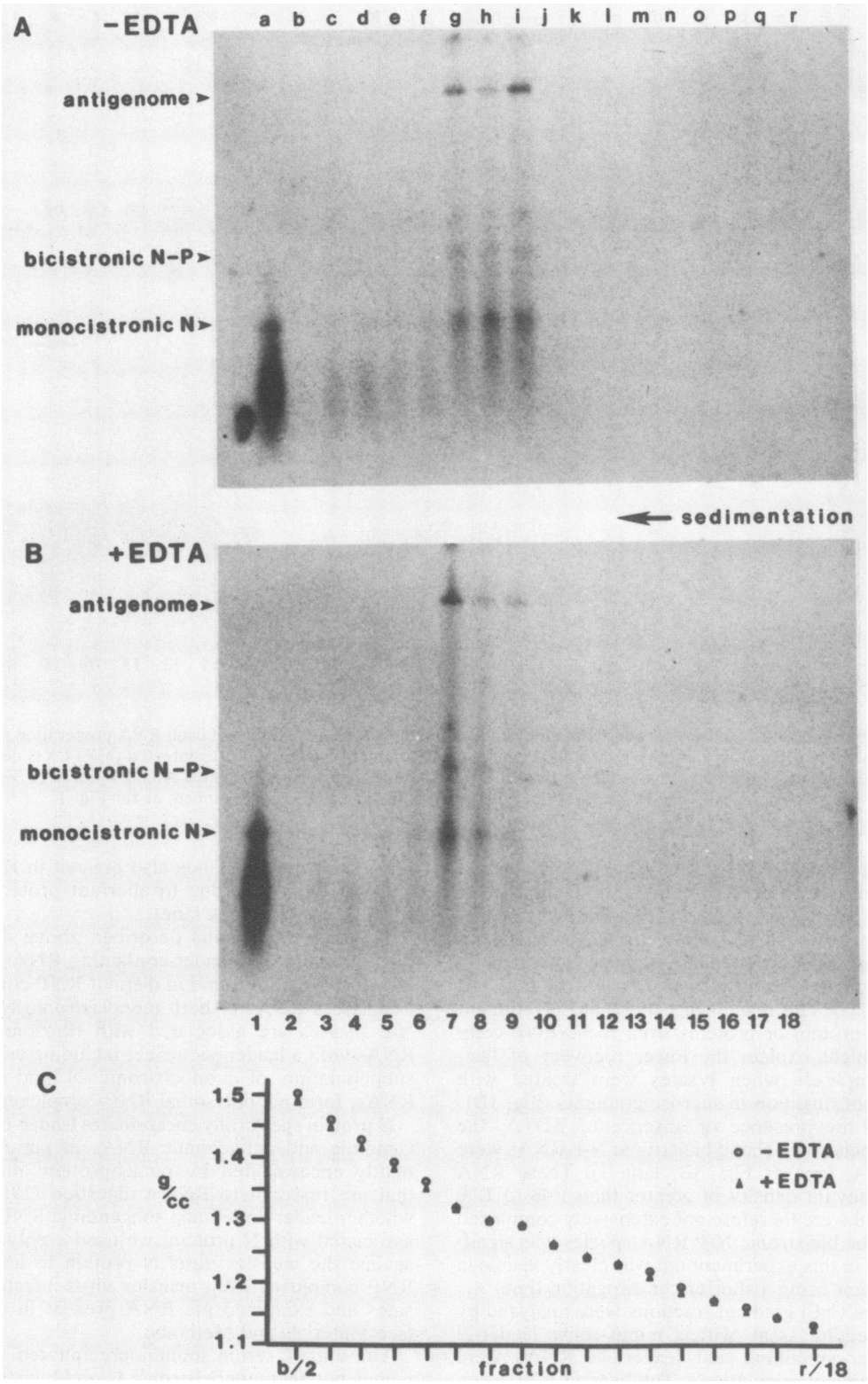


FIG. 3. Equilibrium centrifugation of plus-sense N-containing RNAs. Homogenates from measles virus-infected CV-1 cells were prepared without (A) or with (B) EDTA and centrifuged to equilibrium in 20 to 40% CsCl gradients. One-half of the RNA purified from collected fractions (lanes b to r and 2 to 18) or 1 μg of pelleted RNA (lanes a and 1) was analyzed by Northern blot hybridization with a minus-sense N-specific riboprobe as described in the legend to Fig. 1. (C) Graph depicting CsCl density of each fraction as determined by refractometry.

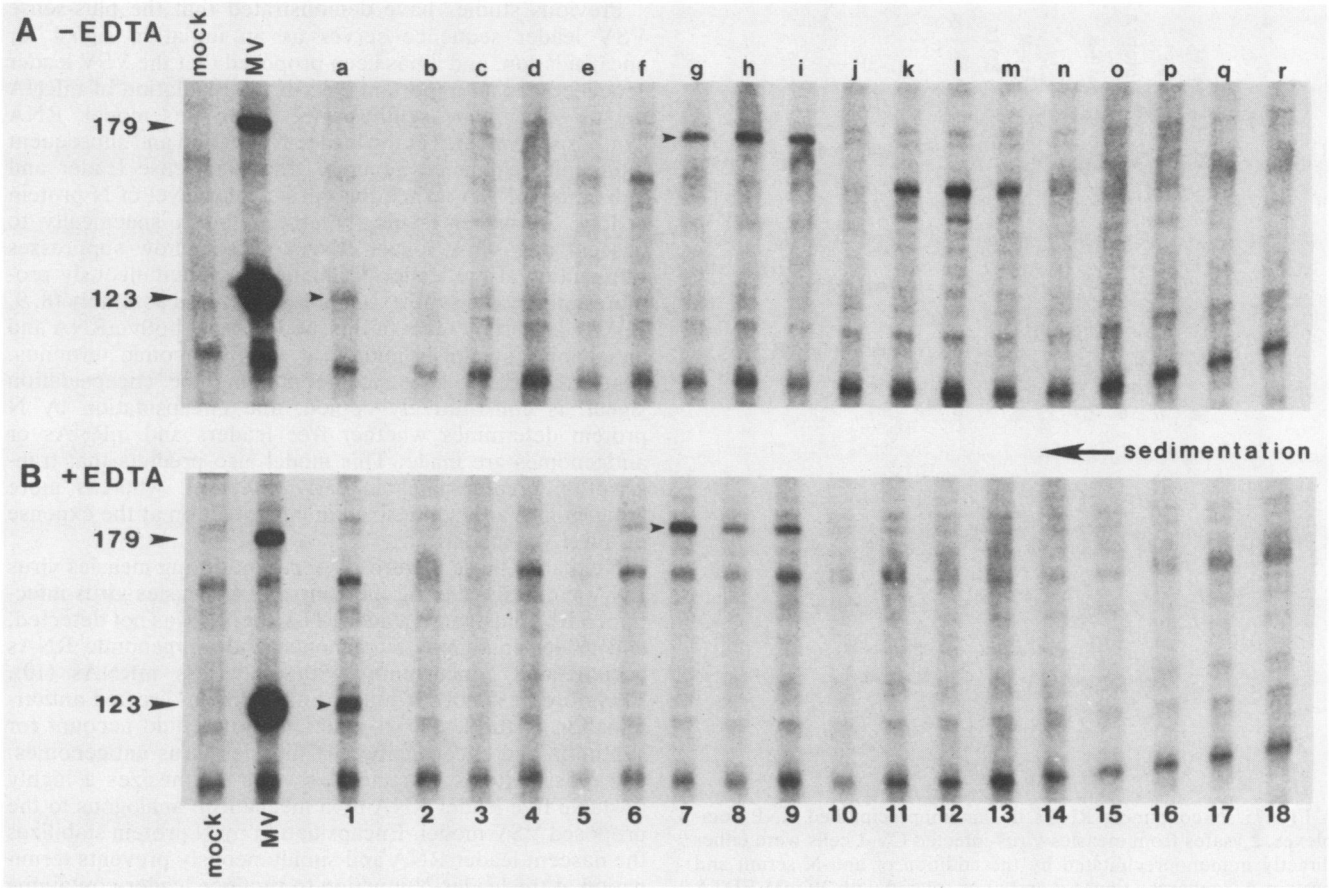


FIG. 4. Equilibrium centrifugation of leader-containing RNAs. One-fourth of each RNA preparation without (lanes b to r) or with (lanes 2 to 18) EDTA treatment recovered from the gradient fractions shown in Fig. 3, 0.5 μg of pelleted RNA (lanes a and 1), or 1 μg of total RNA from uninfected (mock) or measles virus-infected (MV) CV-1 cells was analyzed by the RNase protection assay as described in the legend to Fig. 2.

cipitated subgenomic RNAs were not associated with translational complexes, since the cell lysates were not treated with cycloheximide, which maximizes recovery of intact polysomes. Indeed, disrupting polysomes with EDTA did not significantly affect recovery of either genomic- or subgenomic-length RNPs (Fig. 5A; compare lanes e and f). Furthermore, the same antiserum failed to immunoprecipitate N mRNA from lysates of cells transiently expressing a cloned N gene (data not shown). An anti-H serum also failed to immunoprecipitate these complexes, and hybridization with an H-specific DNA probe detected only antigenomes and no subgenomic-length H-containing RNA in the complexes immunoprecipitated by anti-N serum (data not shown). Most importantly, these subgenomic RNPs were resistant to RNase digestion (Fig. 5A, lane g).

The immunoprecipitated RNAs were also examined by the RNase protection assay with a minus-sense leader-N riboprobe. Total RNA recovered in the lysate without immunoprecipitation protected both a 179-nt and a 123-nt fragment (Fig. 5B, lane a), confirming the presence of both leader-containing and leaderless RNAs, respectively. However, RNA in the immunoprecipitated complexes consistently protected only a 179-nt fragment even if the lysates were pretreated with EDTA or RNase (Fig. 5B, lanes b to d).

These observations indicate that N protein interacts specifically with both leader-containing subgenomic and antigenomic RNAs despite the different lengths of these RNA species. Since N protein does not associate with leaderless RNAs, these results strongly suggest that the leader sequence serves as a signal for encapsidation by N protein.

DISCUSSION

This study demonstrates that measles virus synthesizes two functionally distinct classes of subgenomic N-containing plus-sense RNAs. The first class consists of monocistronic N and bicistronic N-P RNAs that lack a leader sequence. The association of leaderless RNAs with host ribosomes indicates these species function as mRNAs (Fig. 1 and 2). Since only the first cistron of a measles virus bicistronic P-M RNA species is translated (35), leaderless monocistronic N and bicistronic N-P RNA species are most likely translated as a source of N protein. The second class of subgenomic N-containing RNAs consists of monocistronic N and bicistronic N-P species that have a 5′-terminal leader sequence. These leader-containing RNAs are encapsidated by N protein to form nonpolysomal, RNase-resistant RNP complexes which, apart from RNA lengths, are indistinguishable from antigenomic RNP complexes (Fig. 3 to 5). Whether these subgenomic RNP complexes serve any role during an infection remains unknown.

The identification of these two disparate classes of subgenomic N-containing RNAs offers insight into how measles virus might provide for specific encapsidation of antige-

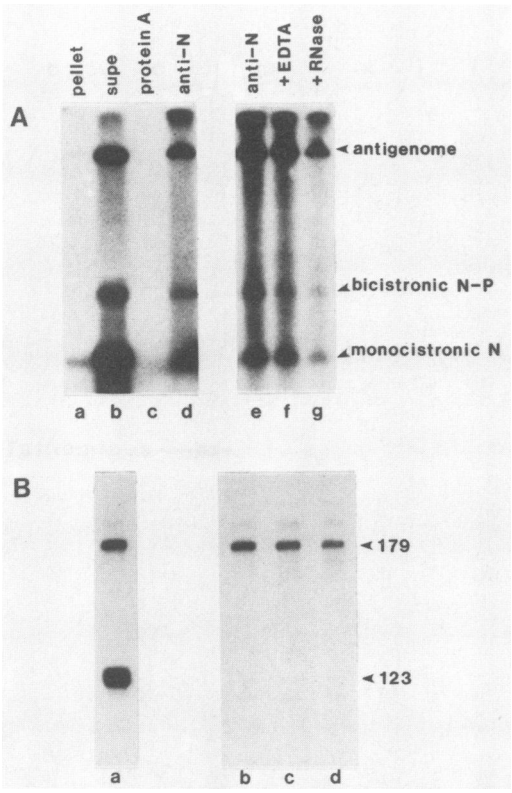


FIG. 5. N-containing RNAs in immunoprecipitated RNP complexes. Lysates from measles virus-infected CV-1 cells were either directly immunoprecipitated by the addition of anti-N serum and protein A-Sepharose (lanes d and e) or treated with 10 mM EDTA (lane f) or 10 μg of RNase A per ml for 20 min (lane g) before immunoprecipitation with the same antiserum. RNA was purified from immune precipitates by 4 M guanidine thiocyanate as described in Materials and Methods. (A) One-half of the recovered RNA was analyzed by Northern blot hybridization with a minus-sense N-specific riboprobe. Lanes: a and b, RNA recovered from the pellet and supernatant, respectively, of the same lysate sample centrifuged in the absence of both antiserum and protein A-Sepharose; c, RNA recovered from the pellet of a sample precipitated by protein A-Sepharose alone. (B) The remainder of the RNA recovered from the immune precipitates was analyzed by the RNase protection assay as described in the legend to Fig. 2. Lanes a, b, c, and d in panel B correspond to lanes b, e, f, and g in panel A.

nomes but not mRNAs and raises questions about how synthesis of these two classes of RNAs is regulated. By RNase protection analysis, the only apparent sequence difference between these two functional classes of RNAs is the presence or absence of the leader sequence (Fig. 1 to 4 and data not shown). Also, we have recently characterized a full-length cDNA derived from a leader-containing polyadenylated N-P RNA species. Other than possessing a 5′-terminal leader sequence, the sequence of this RNA species appeared identical to those of leaderless N-P RNAs (10). Therefore, the initiation site for specific encapsidation of measles virus RNAs is either in the plus-sense leader sequence itself or in the combination of the leader and 5′ N sequences together. Once encapsidation has initiated, this process likely proceeds by cooperative, non-sequence-specific assembly of structural proteins onto the leader-containing RNA, similar to the encapsidation mechanism proposed for tobacco mosaic virus (38) and the rhabdovirus vesicular stomatitis virus (VSV) (8).

Previous studies have demonstrated that the plus-sense VSV leader sequence serves as an initiation signal for encapsidation, and it has been proposed that the VSV leader sequence has an important role in the regulation of mRNA versus antigenome synthesis (8, 9). In this model, RNA synthesis terminates at the leader-N junction and subsequent intergenic regions to generate free plus-sense leader and individual mRNAs when the intracellular level of N protein is low. At higher levels, N protein binds specifically to nascent plus-sense leader RNA and somehow suppresses termination at the leader-N junction to simultaneously promote antigenome synthesis and nucleocapsid assembly (8, 9, 21). An important tenet of this model is that both mRNA and antigenome synthesis initiate at the 3′ genomic terminus. Thus, the leader sequence, containing the encapsidation signal, is constitutively copied, and encapsidation by N protein determines whether free leaders and mRNAs or antigenomes are made. This model also predicts that transcription predominates in early infection, whereas more antigenomes are synthesized in late infection at the expense of mRNA synthesis.

Neither of these features is apparent during measles virus RNA synthesis. During the course of a measles virus infection, a free plus-sense leader RNA species was not detected, and leader-containing subgenomic and antigenomic RNAs accumulated concurrently with leaderless mRNAs (10). Therefore, it is not obvious how N protein-directed antitermination at the leader-N junction alone could account for synthesis and encapsidation of measles virus antigenomes. One possibility is that measles virus synthesizes a highly unstable free leader RNA by a mechanism analogous to the proposed VSV model. Encapsidation by N protein stabilizes the nascent leader RNA and simultaneously prevents termination at the leader-N junction to produce leader-containing RNAs, including antigenomes. This hypothesis is based largely on the VSV model and implies that mRNA and antigenome syntheses are distinguished at the level of termination. Alternatively, leaderless and leader-containing measles virus RNAs might be synthesized by enzymatically distinct processes that are unrelated to RNA encapsidation. In this case, encapsidation requires a leader sequence, but encapsidation is not required to antiterminate at the leader-N junction to produce leader-containing RNAs. A third possibility is that synthesis of leader-containing RNAs and encapsidation are not causally related but are linked to a common regulatory mechanism. Thus, leader-containing RNAs are synthesized only under conditions that allow concomitant encapsidation.

Although we cannot rule out any of these possibilities, the first hypothesis is least compatible with the available data. Encapsidated leader-containing N and N-P RNAs are nearly as abundant as antigenomes (Fig. 1 to 5). If encapsidation by N protein were required for stabilization of a putative free leader RNA to produce leader-containing RNAs, antitermination would have to occur very efficiently at the leader-N junction yet regularly fail at the subsequent N-P and P-M junctions in order to generate these encapsidated subgenomic RNA species without producing a detectable encapsidated free leader RNA. On the other hand, cycloheximide preferentially blocked accumulation of leader-containing and antigenomic RNAs but not leaderless monocistronic and bicistronic RNAs (10). This observation could be explained by either the second or third hypothesis. Notably, synthesis and encapsidation of Sendai virus antigenomes might not be completely explained by N protein-directed antitermination at the leader-N junction, since nucleoprotein alone was

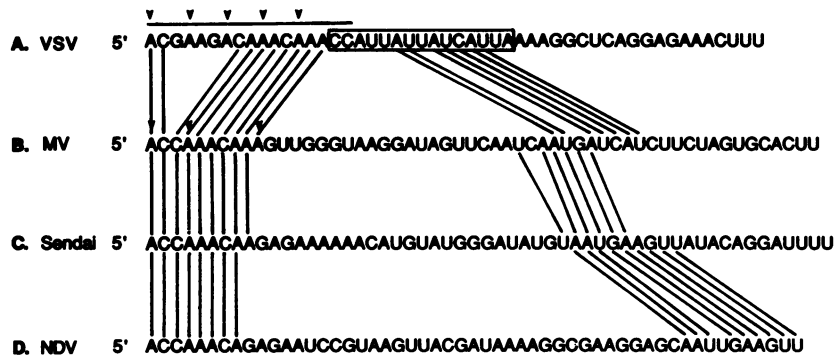


FIG. 6. Sequence comparison of measles virus, VSV, Sendai virus, and Newcastle disease virus leaders. Leader sequences of VSV (13), measles virus (MV; 6, 14), Sendai virus (Sendai; 28), and Newcastle disease virus (NDV; 20) are shown in the plus sense. The A residues postulated to serve as a signal for encapsidation of the VSV leader by N protein are indicated by arrowheads (8), the proposed polymerase entry site is overlined (17), and the sequence contacted by the phosphoprotein involved in RNA synthesis is boxed (18). The sequence homology discussed in the text is indicated by parallel lines and arrowheads.

insufficient to initiate antigenome encapsidation (3) and a recent study revealed unencapsidated leader-containing RNAs in Sendai virus-infected cells (32).

An important question that remains is which sequence(s) in the measles virus leader actually directs RNA encapsidation. For VSV, the encapsidation signal has been postulated to reside in the purine-rich 5'-terminal 14 nt of the plus-sense leader, which contains an A residue at every third position (8, 13; Fig. 6A, arrowheads). Interestingly, there is significant homology between the 5'-terminal sequences of the VSV and measles virus leaders, although the latter contains only three A residues that correspond to the putative VSV encapsidation signal (Fig. 6B, arrowheads). In addition, the two regions in the VSV leader sequence suggested to be important for polymerase initiation and contact with phosphoprotein also share intriguing similarities to sequences in the measles virus leader (17, 18; Fig. 6A and B, respectively). These similarities extend to the leader sequences of two other paramyxoviruses, Sendai virus and Newcastle disease virus (20, 28; Fig. 6C and D, respectively). Notably, these two regions of homology are separated by variable lengths of purine-rich sequences in the paramyxovirus leader sequences.

In summary, these data provide further insights into the RNA synthesis process of measles virus and suggest a mechanism for how measles virus differentially generates RNA products intended for protein synthesis and genome replication.

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