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Cloning of DNA complementary to the measles virus mRNA encoding nucleocapsid protein

(viral clone identification/subacute sclerosing panencephalitis)

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ABSTRACT **Double-stranded cDNA synthesized from total poly(A)-containing mRNA, extracted from monkey cells infected with measles virus, has been inserted into the *Pst* cleavage site of *Escherichia coli* plasmid pBR322 and cloned. A clone containing measles virus DNA sequences was identified by hybridization to a measles virus-specific ^{32}P-labeled cDNA probe prepared from the mRNA of measles virus-infected cells. Cellular sequences in the probe were neutralized by prehybridization with an excess of unlabeled mRNA from uninfected monkey cells. The insert of cloned cDNA isolated contains 1420 base pairs, as shown by agarose gel electrophoresis and electron microscopy. The size of the mRNA complementary to this cloned cDNA is 1750 nucleotides, as determined by the reverse Southern technique. The cloned DNA fragment was further identified as the reverse transcript of the mRNA coding for the nucleocapsid protein of measles virus on the basis that the major cell-free translation product of mRNA selected by hybridization to the cloned DNA comigrated with the nucleocapsid protein and was immunoprecipitated by measles virus-specific antibodies. Subsequently, the cloned DNA was used to detect specific measles virus sequences in the poly(A)-RNA extracted from brain autopsy material from a patient with subacute sclerosing panencephalitis. The cloned DNA can thus be used as a probe to study the structure and expression of the measles genome, and in particular, to study diseases of the central nervous system in which persistent infection with measles virus has been implicated.**

Measles virus is a complex virus that interacts with its host cell in either an acute or a persistent infection. The measles viroid consists of six structural proteins (1–4) and a single-stranded RNA (M_r 6 × 10^6) (1, 5) that could encode as many as 10–15 average-sized proteins. Although the measles virus genome generally is regarded as a negative-strand RNA (6), recent evidence suggests that also viral plus-strand RNA is encapsidated within measles nucleocapsid preparations (unpublished observations). Six virus-specific messenger RNAs, differing in size, have been recognized in monkey cells infected with measles virus in the presence of actinomycin D (7). Poly(A)-containing mRNAs synthesized by measles-infected monkey cells have been purified and translated *in vitro*. The proteins synthesized in an mRNA-dependent reticulocyte cell-free system subsequently were identified as authentic measles polypeptides by electrophoretic and immunologic techniques as well as by analysis of their tryptic peptide patterns (8). Thus, it is now possible to examine measles virus-specific gene expression during the course of infection directly and to determine whether acute versus persistent infections can be correlated with differential patterns of gene expression.

A powerful approach to studying differential gene expression in eukaryotic cells and in cells infected with virus involves the development of nucleic acid hybridization probes that are highly radioactive and gene specific.

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In this report we describe the isolation and characterization of a recombinant plasmid that contains most, if not all, of the nucleic acid sequence encoding measles nucleocapsid protein. This clone was obtained by the insertion of a reverse transcript of poly(A)-containing mRNA from monkey cells acutely infected with measles virus.

A considerable body of information now implicates persistent infection of measles virus in the etiology of at least one human neurological disease, subacute sclerosing panencephalitis (SSPE) (9–13). We also report that our recombinant plasmid provides a nucleic acid probe sensitive enough to detect measles virus gene sequences in poly(A)-containing mRNAs isolated from brain tissue taken at autopsy of a patient with SSPE.

MATERIALS AND METHODS

Cells and Viruses. The CV-1 line of African green monkey kidney cells was obtained from Flow Laboratories (Rockville, MD). The cells were grown in Eagle's medium supplemented with 10% calf serum. The Edmonston strain of measles virus, obtained from B. Fields (Harvard Medical School) was plaque-purified twice in CV-1 cells. A stock was prepared by infecting CV-1 cells at a multiplicity of infection of 1/1000. After the development of a marked cytopathic effect, virus was harvested from freeze–thaw lysates of the cells. Cell debris was removed by centrifugation at 2000 × *g* for 10 min at 4°C, and supernatants containing 3–6 × 10^6 plaque-forming units/ml, as titrated in Vero African green monkey cells, were stored at −90°C.

Preparation of RNA. Cytoplasmic RNA was extracted from 6 × 10^8 CV-1 cells 24 hr after infection with plaque-purified measles virus at 0.1–0.5 plaque-forming units/cell. RNAs were purified by successive phenol/chloroform/isoamyl alcohol extractions followed by LiCl precipitation (8). Poly(A)-mRNAs then were purified by two cycles of oligo(dT)-cellulose chromatography.

Synthesis of Double-Stranded cDNA. cDNA was synthesized from poly(A)-RNA purified from measles virus-infected cells in a 200-μl reaction mixture containing: Tris·HCl (50 mM, pH 8.3); $MgCl_2$ (9 mM); dithiothreitol (0.5 mM); KCl (60 mM); sodium pyrophosphate (4 mM); dTTP, dCTP, dATP, and dGTP (1 mM each); [^{3}H]dATP (New England Nuclear, 0.5 mM, 0.4 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels); $(dT)_{12-18}$ (5 μg/ml); mRNA (100 μg/ml); and reverse transcriptase (105 units) obtained from J. Beard, Life Sciences, St. Petersburg, FL. The mixture was incubated for 1 hr at 37°C and the reaction was terminated by addition of NaOH (0.3 M). After 20 min at 70°C, the mixture was neutralized and deproteinized by phenol extraction. The supernatant was desalted by a 4-min centrifugation through a 3-ml Sephadex G-50 column (Isolabs) equilibrated with buffer containing $NaDodSO_4$ (0.1%), NaCl (0.1 M),

Abbreviations: SSPE, subacute sclerosing panencephalitis; bp, base pairs; DBM, diazobenzoyloxymethyl.

and Tris·HCl (10 mM, pH 8). The excluded fraction was adjusted to 0.3 M sodium acetate and precipitated overnight at −20°C with 2.5 vol of ethanol. The yield was 2.4 μg. The cDNA was then subjected to second-strand synthesis in a reaction mixture (200 μl) containing: Tris·HCl (50 mM, pH 7.8); $MgCl_2$ (9 mM); dithiothreitol (0.5 mM); four deoxynucleotide triphosphates (1 mM); [α-^{32}P]CTP (New England Nuclear, diluted to 0.7 Ci/mmol) and reverse transcriptase (105 units). After 4 hr the reaction was terminated by addition of $NaDodSO_4$ (0.5%) and EDTA (1 mM), and duplex cDNA was recovered as described above. The yield of double-stranded cDNA was 4.8 μg. To remove single-stranded cDNA regions remaining in the preparation, 2 μg of double-stranded cDNA was digested for 20 min at 37°C with S1 nuclease (Boehringer, 50 units), in a 300-μl reaction mixture containing: sodium acetate (0.03 M, pH 4.5); NaCl (0.25 M) and $ZnSO_4$ (1 mM). After deproteinization with phenol, double-stranded cDNA was recovered as described above.

Homopolymer Tailing and Hybridization. pBR322 DNA (1 μg) digested with endonuclease *Pst* I and S1 nuclease-digested double-stranded cDNA (200 ng) were elongated by addition of $(dG)_{8-12}$ and $(dC)_{10-15}$ tails, respectively. The reaction mixtures (50 μl) contained: sodium cacodylate (0.14 M, pH 7.6); Tris·HCl (0.03 M); dithiothreitol (0.1 mM); $CaCl_2$ (1 mM); [^{32}P]dGTP or dCTP (40 μM, 2 Ci/mmol); and calf thymus terminal deoxynucleotidyltransferase (10 units, P-L Biochemicals). After 90 sec at 37°C, the reactions were terminated by addition of $NaDodSO_4$ (0.1%). Both DNAs (pBR322 and cDNAs) were mixed together and recovered as described above. The mixture was dissolved in 100 μl of a buffer containing Tris·HCl (10 mM, pH 7.8), NaCl (0.1 M), and EDTA (1 mM); heated for 10 min at 70°C; and then incubated at 48°C for 2 hr to allow for hybridizing of the partially single-stranded oligo(dG) and oligo(dC) stretches. After hybridization, the DNA was again precipitated and washed with ethanol and air-dried.

Transformation and Colony Hybridization. All experiments were carried out in the P3 facility of the Weizmann Institute of Science in accordance with the National Institutes of Health *Guidelines for Research Involving Recombinant DNA Molecules*. *Escherichia coli* strain HB101 was transformed with recombinant pBR322 DNA as described (14). Tetracycline-resistant clones were replated on LB agar plates containing tetracycline (12.5 μg/ml) and then were transferred to nitrocellulose filters for colony hybridization using a measles-specific [^{32}P]cDNA probe prepared from poly(A)-RNA from infected cells (15).

Isolation and Purification of Plasmid DNA. Clones were grown to an OD_{600} of 0.6 in LB broth containing tetracycline. Chloramphenicol (100 μg/ml) was added and incubations were continued overnight. The cells were collected by centrifugation, washed twice with Tris·HCl (1.0 mM, pH 7.8)/EDTA (1 mM) and suspended in the same buffer (25 ml/liter of cell culture). Lysozyme (4 mg/ml) was added, and the suspensions were incubated on ice for 20 min. $CsCl_2$ (0.9 g/ml) then was added and the solutions were centrifuged at 15,000 × *g* for 1 hr. After removal of the upper protein-containing layers, supernatants were supplemented with ethidium bromide (0.3 mg/ml), their densities were adjusted to 1.6 g/ml, and then they were centrifuged in a Beckman type 65 rotor at 40,000 rpm for 48 hr at 20°C. Supercoiled plasmid DNAs were removed, ethidium bromide was extracted with isopropanol, and the DNAs were diluted with 3 vol of water and precipitated with 2.5 vol of ethanol.

Plasmid DNAs were digested with restriction endonucleases (New England BioLabs) according to the supplier's instructions. The resulting DNA fragments were fractionated according to size by electrophoresis in agarose gels (1.5%) in buffer containing Tris/acetate (20 mM, pH 7.8) and EDTA (1 mM).

Selection of Measles Virus-Specific RNA. Selection of RNA was carried out according to Ricciardi *et al.* (16). Cloned DNA, linearized with *Eco*RI (5–10 μg) was denatured (100°C, 5 min) and applied to 1-cm-square nitrocellulose filter pads presoaked in 0.9 M NaCl/0.09 M sodium citrate. The nitrocellulose filter was air-dried and then baked at 75°C for 2 hr under reduced pressure. Hybridization mixtures (100 μl) consisted of 10 μg of poly(A)-RNA, 10 mM 1,4-piperazinediethanesulfonic acid (pH 6.4), 0.4 M NaCl, and 65% (wt/vol) formamide. Hybridization incubations were carried out at 50°C.

RESULTS

Construction and Identification of cDNA Clones. Double-stranded cDNA prepared from poly(A)-containing RNA extracted from measles virus-infected cells consisted of a heterogenous population of molecules with a distinct band that migrated to a position on the electrophoretogram corresponding

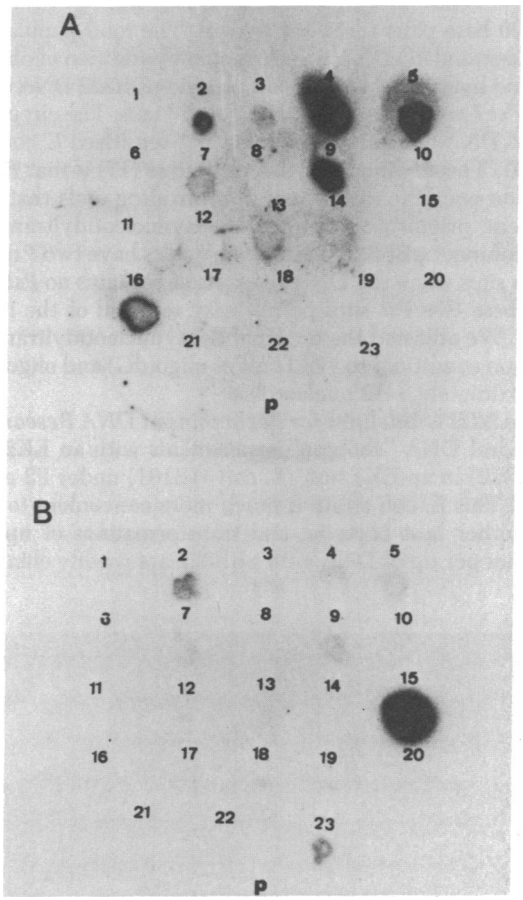

FIG. 1. Detection of measles virus-specific clones. Transformants were transferred onto nitrocellulose papers and hybridized with ^{32}P-labeled cDNA probes (2 × 10^5 cpm/ml) prepared from poly(A)-RNA from uninfected cells (*A*) or poly(A)-RNA from acutely infected cells (*B*). The latter probe was incubated, prior to hybridization, with a 100-fold excess (10 μg) of poly(A)-RNA from uninfected cells. The incubation was performed at 68°C for 4 hr in a 100-μl reaction mixture containing 0.27 M sodium citrate (pH 7.2), 0.9 M NaCl, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 150 μg of sonicated salmon sperm DNA. After hybridization filters were washed and autoradiographed.

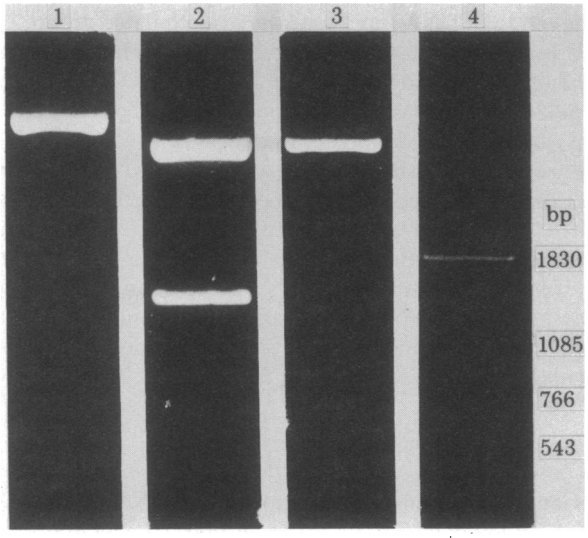

FIG. 2. Sizing of measles virus DNA clone. DNAs were electrophoresed on 1.5% agarose slab gels and stained with ethidium bromide. Lane 1, clone 15 plasmid digested with *Eco*RI; lane 2, clone 15 plasmid digested with *Pst* I; lane 3, pBR322 digested with *Pst* I; lane 4, simian virus 40 digested with *Hin*fI.

to 1500 base pairs (data not shown). The total population of double-stranded cDNA was elongated by addition of oligo(dC) tails and hybridized with *E. coli* plasmid pBR322 DNA cleaved with *Pst* I and extended with oligo(dG) tails. The circularized hybrid DNA was introduced into Ca^{2+}-sensitized *E. coli* strain HB101. The advantage of this technique (17) is that *Pst* I restriction endonuclease leaves 3′ protruding ends that are an efficient primer for terminal deoxynucleotidyltransferase. Recombinant pBR322 plasmid molecules have two *Pst* recognition sites when the DNA insert itself contains no *Pst* I sites, and these two *Pst* sites permit easy removal of the inserted DNA. We adjusted the terminal deoxynucleotidyltransferase reaction conditions to yield tails of oligo(dG) and oligo(dC) of approximately 8–12 nucleotides.

The *NIH Guidelines for Recombinant DNA Research* permits viral DNA "shotgun" experiments with an EK2 vector (pBR322) in an EK1 host (*E. coli* HB101) under P3 containment. This *E. coli* strain is much more convenient to handle than other host bacteria, and transformations of up to 10^7 colonies per μg of DNA with pBR322 are readily obtained by

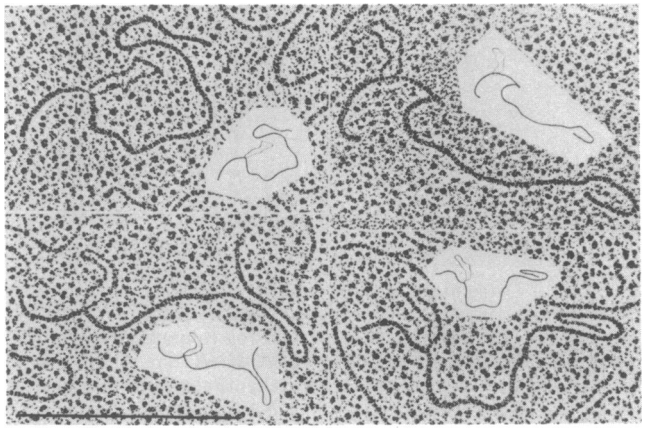

FIG. 3. Electron microscopy of heteroduplexes between pBR322 and clone 15 DNAs. pBR322 and clone 15 DNAs (1 μg of each) were linearized with *Eco*RI, denatured, and annealed (18). *Insets* show interpretive drawings. Bar indicates 0.5 μm.

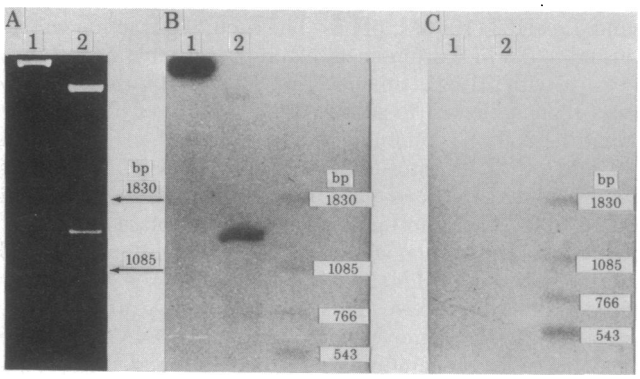

FIG. 4. Characterization of clone 15 DNA by the Southern technique. Clone 15 DNA, digested with *Eco*RI (lanes 1) and *Pst* I (lanes 2), was fractionated on a 1.5% agarose slab gel stained with ethidium bromide (*A*) and subsequently transferred onto nitrocellulose paper (19). The nitrocellulose paper was hybridized with [^{32}P]cDNA made of poly(A)-RNA from measles virus-infected cells (*B*) or uninfected cells (*C*). ^{32}P-Labeled simian virus 40 *Hin*fI fragments were used as internal markers.

standard procedures. Transformants resistant to tetracycline (15 μg/ml) were isolated and measles virus-containing sequences were identified by a recently developed blocking method (unpublished data), adapted for screening clones that contain measles virus DNA sequences. Cloned DNA from individual colonies was transferred to nitrocellulose filters and assayed with a measles virus-specific hybridization probe, prepared by transcribing poly(A)-containing RNA from measles virus-infected cells into [^{32}P]cDNA. This cDNA therefore contained both virus and host mRNA sequences. [^{32}P]cDNA sequences derived from host cell mRNAs were removed from the preparation by blocking these sequences during prehybridization with a 100-fold excess of poly(A)-RNA from uninfected cells.

Fig. 1*A* shows the hybridization patterns of cloned DNA contained by transformants that had been transferred to nitrocellulose filters. The hybridization probe in this experiment was ^{32}P-labeled cDNA prepared from poly(A)-containing RNA from uninfected cells. Five clones gave a positive hybridization signal and were considered to contain plasmids with DNA sequences derived from African green monkey cells. A duplicate filter of transformant colonies was hybridized with a measles virus-enriched probe, obtained by the blocking procedure (Fig. 1*B*). Only one clone (no. 15) reacted with this probe and thus appeared to contain a virus-specific DNA sequence. This single clone represents only about 10% of the total number of identifiable cellular clones, in good agreement with the estimated number of viral sequences in RNA from infected cells. Subsequent transformation experiments confirmed this finding. Thus, the cDNA probe, although prepared by oligo(dT) priming of the poly(A)-RNA template, faithfully reflects the distribution of sequences in the infected cells. However, it failed to detect sequences present in a few tetracycline-resistant ampicillin-sensitive plasmids, in spite of the fact that all of them were found to contain DNA inserts (data not shown). It is plausible, therefore, to assume that the smaller inserts produce weaker hybridization signals.

Characterization of Measles Clone 15. Digestion of clone 15 DNA with *Pst* I (Fig. 2, lane 2) generated two fragments. One corresponded in size to linear pBR322 DNA, such as was obtained by digestion with *Eco*RI (lane 1); a second fragment consisted of 1420 base pairs (bp). The size of the *Pst* fragment was further confirmed by electron microscopic studies. Fig. 3 shows electron micrographs of heteroduplex molecules formed

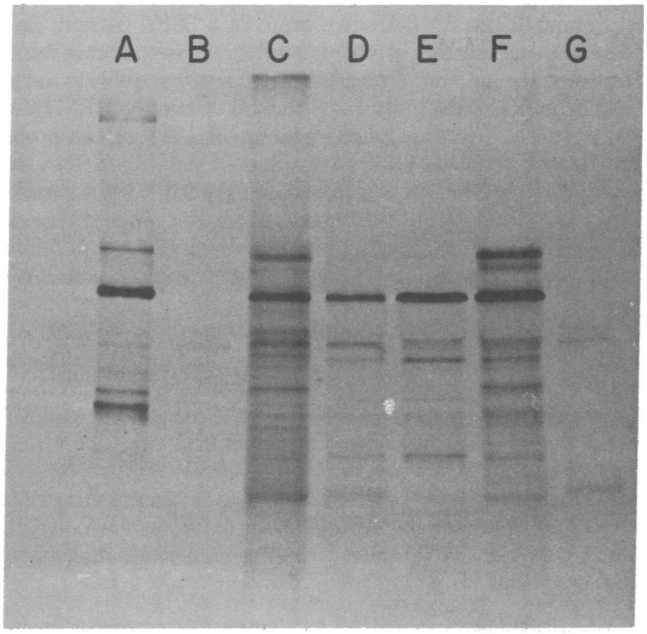

FIG. 5. Products of the *in vitro* translation of RNA selected by hybridization to pBR-MVN (clone 15). [^{35}S]Methionine-labeled polypeptides synthesized in reticulocyte cell-free systems (8) were fractionated on a NaDodSO$_4$/10% polyacrylamide gel. The dried gel was fluorographed at −80°C (20). Immunoprecipitation was carried out with anti-measles guinea pig serum as described (8). Lane A, [^{35}S]methionine-labeled nucleocapsid purified from cells acutely infected with measles virus (21). Lanes B–D, translation products under direction of: no added RNA (B), total cytoplasmic poly(A)-RNA (0.5 μg) isolated 24 hr after infection (8) (C), RNA selected by hybridization of total cytoplasmic poly(A)-RNA to immobilized clone 15 DNA (D). Lanes E–G, immunoprecipitates of: the RNA of lane D (E), the RNA of lane C (F), and RNA hybridized to immobilized pBR322 DNA (G).

by reassociation of pBR322 and clone 15 DNAs, both linearized by digestion with *Eco*RI, which does not cut the insert (Fig. 2, lane 1). The length of the inserted measles virus sequence appears in these micrographs to be 1420 ± 20 bp, in excellent agreement with the length determined by agarose gel electrophoresis. Only the undigested clone 15 DNA and the 1420-bp fragment hybridized with ^{32}P-labeled measles virus-specific cDNA probe (Fig. 4*B*) but not with the probe from uninfected cells (Fig. 4*C*). This agrees with the colony hybridization data (see above) and again suggests that this clone contains measles virus-specific DNA sequences.

Correlation of the Cloned Measles DNA with a Specific Viral Gene Product. Clone 15 DNA was digested with *Eco*RI, immobilized onto nitrocellulose filters, and hybridized with poly(A)-containing RNA from measles virus-infected cells. RNA was then eluted from the filters and translated in a mRNA-dependent rabbit reticulocyte cell-free system. As shown in Fig. 5, lane D, the major cell-free translation product comigrated with the 60,000-dalton nucleocapsid measles virus polypeptide, whereas other measles virus-specific polypeptides, such as the 70,000-dalton P protein and the 37,000-dalton M protein (21), were missing. Furthermore, this translation product immunoprecipitated with anti-measles antibodies (Fig. 5, lane *E*). As expected, no translatable RNAs were eluted from filters containing control pBR322 DNA (Fig. 5, lane G). We conclude from these results that the cDNA inserted in the recombinant plasmid contained in clone 15 is specific for the measles virus nucleocapsid protein. Therefore, we designate it clone pBR-MVN (measles virus nucleocapsid).

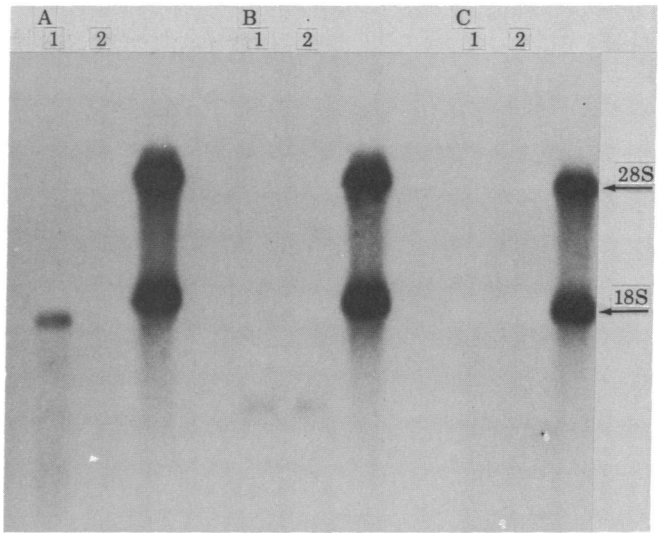

FIG. 6. Sizing of measles virus nucleocapsid mRNA by hybridization of clone pBR-MVN (clone 15) to total poly(A)-RNA from cells acutely infected with measles virus. Poly(A)-RNA (2 μg) from measles virus-infected cells (lanes 1) and from uninfected cells (lanes 2) was fractionated under denaturing conditions (19) in a 1.5% agarose gel. The RNA was transferred to DBM paper and hybridized with "nick-translated" (22) plasmid [^{32}P]DNA. (*A*) pBR-MVN (clone 15); (*B*) pBR322 with an insert of monkey sequences; (*C*) pBR322. ^{32}P-Labeled 18S and 28S ribosomal RNA were used as markers.

DISCUSSION

The cloning of a cDNA that appears to specify most, if not all, of the nucleotide sequences coding for a measles virus nucleocapsid protein is reported here. The double-stranded cDNA was inserted into the *Pst* site of *E. coli* plasmid pBR322 DNA by hybridization of "tailed" DNAs. The recombinants were then used to transform *E. coli*. A clone containing measles virus DNA sequences (clone 15) was identified by colony hybridization using a [^{32}P]cDNA probe, rendered measles virus-specific by blocking cDNA that arose through reverse transcription of host cell (monkey) mRNA sequences. The advantage of this probe is that it recognizes viral nucleic acid sequences specifically and thus directly discriminates among clones containing plasmids with host virus DNA inserts. Furthermore, to ascertain the nature of measles virus RNA homologous to the cloned DNA, we utilized the techniques of RNA selection by hybridizing nitrocellulose filters containing specific immobilized DNAs (16). Translation *in vitro* of the measles virus RNA purified by this method has shown unambiguously that the cloned double-stranded cDNA, contained within clone 15, encodes at least a portion of, and possibly most of, the measles virus nucleocapsid protein (8, 21). The major radioactive polypeptide product synthesized in a cell-free reticulocyte translation system comigrated with authentic nucleocapsid polypeptide and was the only polypeptide specifically immunoprecipitated by antisera directed against measles virus.

Two other minor radioactive polypeptides were also synthesized, although their origin is not known. These polypeptides might be encoded by smaller RNAs that are also hybridized to the cloned DNA. The number and size of measles virus-specific mRNAs have recently been determined by a technique similar to the one described above. This technique consisted of the fractionation of poly(A)-containing RNAs from measles virus-infected cells on agarose gels under denaturing conditions and their transfer onto diazobenzoyloxymethyl (DBM) paper. The viral sequences then were detected by hybridization with a ^{32}P-labeled cDNA probe prepared from total poly(A)-RNA from measles virus-infected cells rehybridized with an excess

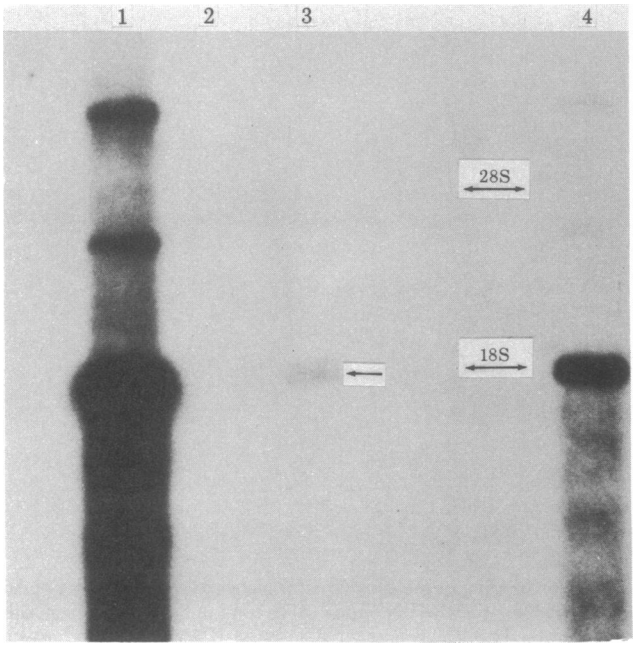

FIG. 7. Measles virus sequences in RNA extracted from the brain of a SSPE patient after autopsy. Poly(A)-RNA was fractionated, transferred to DBM paper, and hybridized with measles virus-specific [^{32}P]cDNA (4×10^8 cpm/μg; 2×10^7 cpm). This probe gave a strong hybridization signal with all known measles virus-specific mRNAs derived from infected cells (unpublished data). RNA from SSPE brain failed to show any hybridization signal, even after prolonged exposure. The same DBM blot was subsequently rehybridized with nick-translated ^{32}P-labeled pBR-MVN (clone 15). Poly(A)-RNA was from: lane 1, measles virus-infected cells (2 μg); lane 2, uninfected cells (2 μg); lane 3, brain of a SSPE patient (2 μg); lane 4, measles virus-infected cells (0.2 μg). An arrow points to the position of a weak band in lane 3.

of unlabeled poly(A)-RNA from uninfected cells. By this technique, the most abundant virus-specific mRNAs were estimated to be 1750 nucleotides in length; this is the predominant mRNA species hybridized with the cloned pBR-MVN DNA (Fig. 6A, lane 1) and therefore appears to encode an authentic measles virus protein, the nucleocapsid protein. The molecular weight of measles virus nucleocapsid protein, which is approximately 60,000 as measured by $NaDodSO_4$ gel electrophoresis, is in good agreement with the coding capacity of the measles virus mRNA species assigned to this protein. It is noteworthy that the cloned double-stranded cDNA sequence of 1420 bp represents almost a full-length transcript of the nucleocapsid protein gene, including a poly(A) tail of 100–200 nucleotides, which is usually present in eukaryotic mRNAs.

Considerable evidence implicates measles or a closely-related virus in a rare degenerative neurological disease, SSPE. The obvious question in the etiology of SSPE is whether the brain represents the target tissue of the virus in which the specific viral RNA is synthesized and expressed or whether it is only an organ destined for deposition of the mature viral particles. Our approach to this question was to search for measles virus-specific sequences in poly(A)-RNA from the brain of deceased SSPE patients. Fig. 7 shows the hybridization of SSPE RNA fractionated under denaturing conditions on agarose gels after transfer to DBM paper and hybridization with a probe prepared from nick-translated ^{32}P-labeled clone pBR-MVN DNA. The only band visible in the fractionated RNA was a band corresponding to the largest species observed in mRNA from infected cells, the species identified as nucleocapsid protein mRNA. This experiment provides direct evidence that measles-related RNA is present in the RNA from a brain of a SSPE patient, and therefore suggests that mRNA is synthesized in the brain itself. However, the amount of measles virus-specific sequences in the RNA from this SSPE brain tissue is small. When the DBM filter shown in Fig. 7 was analyzed by means of a [^{32}P]cDNA probe synthesized from measles virus-infected poly(A)-mRNA directly, no hybridization was observed in the SSPE RNA sample. However, as shown in the figure, when the same filter was subsequently analyzed with [^{32}P]DNA from pBR-MVN (clone 15) a weak but distinct band of measles sequence was detected.

In the course of these cloning experiments, we obtained a variety of measles virus-specific DNA clones, in addition to those that were complementary to pBR-MVN. They probably represent clones specific for proteins of the measles virus family, other than nucleocapsid proteins, which still await identification. We hope that with the aid of the cloned measles DNAs we will be able to detect the whole spectrum of measles virus RNA sequences present in SSPE brain tissue, as well as the molecular forms (RNA or DNA) in which these sequences are maintained and expressed.

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