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Cell-Free Synthesis of Measles Virus Proteins

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Polyadenylated mRNA extracted from cytoplasm of measles virus-infected Vero cells was translated in a cell-free system. Three of the polypeptides obtained corresponded to nucleocapsid protein, phosphoprotein, and membrane protein of measles virions. A fourth polypeptide, present in measles virus-infected cells, could be generated by addition of Vero cytoplasmic extract and was identified as a cleavage product of the nucleocapsid protein.

The isolation of measles virus from the brains of patients with the slowly progressive neurological disease subacute sclerosing panencephalitis and its possible association with multiple sclerosis (1, 7, 19) have stimulated investigations into measles virus RNA synthesis (6, 16). The measles virus genome consists of a nonsegmented single-stranded RNA molecule with a molecular weight of approximately 6×10^6 (16) which sediments as a 50S species. In addition to 50S RNA, two heterogeneous populations of viral RNA which sediment as 18S and 35S species (6) are synthesized in measles virus-infected cells. A recent report has demonstrated that polyadenylated measles virus mRNA consists of seven to nine species (5).

Polyadenylated RNA from measles virus-infected Vero cells was translated in cell-free systems derived from either wheat germ (see Fig. 1, 2, and 3) or rabbit reticulocytes (data not shown). In both systems, three prominent polypeptides which comigrated with authentic virion polypeptides during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1) were observed. The sizes of these three in vitro products corresponded to measles membrane (M) protein, nucleocapsid (NC) protein, and nucleocapsid-associated phosphoprotein (P), which have molecular weights of 38,000 (38K), 60K, and 69K, respectively. Polyadenylated RNA from uninfected cells did not direct the synthesis of these three polypeptides (Fig. 1).

Immunoprecipitation, tryptic peptide analysis, and two-dimensional gel electrophoresis provided further evidence that these three in vitro polypeptides corresponded to authentic measles proteins. The three in vitro polypeptides were immunoprecipitable with measles antiserum, but not with control antiserum when immunoprecipitation was performed as described by Seifried et al. (17) (data not shown). Their identities were also confirmed by tryptic peptide analysis (Fig. 2) or by two-dimensional comparison (data not shown), utilizing isoelectric focusing followed by electrophoresis in the perpendicular direction (12). Tryptic peptide analysis of the 38K product was unsatisfactory owing to the presence of an additional acidic polypeptide of the same electrophoretic mobility synthesized in the reticulocyte translation system. However, authentic M protein shows charge and size properties identical to the 38K product in the two-dimensional system.

A prominent band (44K) was observed to be migrating in the vicinity of actin. The quantity of this band varied with the mRNA preparation; for example, translation directed by uninfected Vero cell mRNA (Fig. 1, lane 1) showed little or no actin. Other uninfected Vero cell mRNA preparations did program the translation of the 48K polypeptide. Tryptic peptide analysis showed the band to be like cellular actin (J. Sprague, L. J. Eron, and J. C. Leavitt, unpublished observations). These observations are analogous to those recently published by Rozenblatt et al. (15) utilizing mRNA from CV-1 cells infected with a different measles virus strain.

No in vitro polypeptides were seen to have electrophoretic mobilities near that of the viral glycoprotein. Other investigators have reported an inability to detect synthesis of glycosylated proteins in the wheat germ system (2, 4, 13). Furthermore, 78K protein was not detected in immunoprecipitates of viral polypeptides synthesized in a reticulocyte cell-free translation system (15).

To insure that the translation products seen were the result of translation of measles virus mRNA, we sedimented polyadenylic acid-se-

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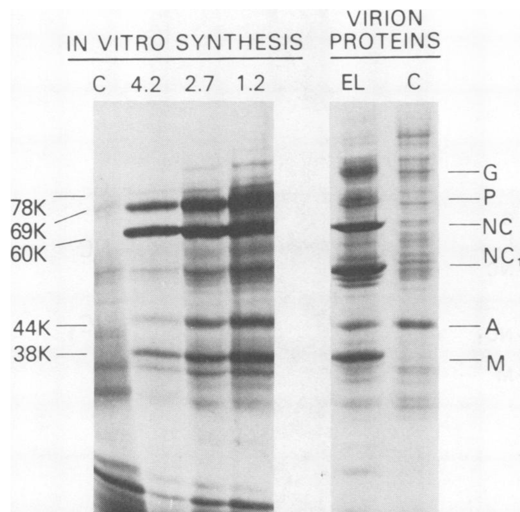

FIG. 1. *Autoradiography of cell-free translation of RNA from measles virus-infected cells at various magnesium concentrations (4.7, 2.7, and 1.2 mM) and from mock-infected Vero cells (C) (1.2 mM Mg^{2+}) and authentic [^{35}S]methionine-labeled polypeptides from disrupted measles virus (EL) or from mock-infected Vero cells (C). Sodium dodecyl sulfate-polyacrylamide (10%) slab gels were prepared and run as described by Maizel (10). The molecular weights indicated on the figure were determined by using adenovirus polypeptides as markers (a gift of H. Westphal). The positions of authentic measles polypeptides are labeled in accordance with published reports (1, 11). A, Actin; NC_1, so labeled because of its relation to NC protein (see text). Wheat germ cell-free translations were performed as described by Roberts and Paterson (14). Rabbit reticulocyte lysate cell-free translations gave a similar pattern and were used for preparation of synthetic polypeptides for two-dimensional electrophoretic analysis, using a [^{35}S]methionine-translation kit (New England Nuclear Corp.) at 1.9 mM Mg^{2+} for a 2-h incubation period. Viral and cellular mRNA's for translation were prepared by extracting the autodigested, pronase-treated (0.2 mg/ml, 37°C, 15 min) postnuclear supernatant with phenol-chloroform-isoamyl alcohol (50:50:1), concentrating the RNA in the aqueous layer by precipitation with 2.5 volumes of EtOH, and selecting for polyadenylic acid-containing mRNA on oligo(dT)$_{12-18}$-cellulose. In each 25-μl reaction mixture, 0.5 to 20 μg of purified RNA was used, depending on optimal concentrations. The total translation products from a 25-μl reaction mixture were diluted in buffer containing 2-mercaptoethanol and sodium dodecyl sulfate, treated at 100°C for 2 min, and electrophoresed. The Edmonston vaccine strain of measles virus was used to infect Vero cells at a multiplicity of 1 PFU/cell. Under these conditions, complete cytopathic effect occurred at 48 h postinfection; labeling (5 μCi of [^{35}S]methionine per ml in serum-free, methionine-free medium [National Institutes of Health media unit]) was for 24 h before harvesting. The virus was purified from washed infected cells by swelling cells in 0.01 M KCl-0.01 M Tris (pH 7.4)-1.5 mM $MgCl_2$ at 4°C, homogenizing in a Dounce homogenizer, and centrifuging out the nuclei at 600 × g for 5 min (postnuclear supernatant). The virus in the postnuclear supernatant was further purified by centrifuging onto a sucrose cushion and then centrifuging to equilibrium on an isopycnic sucrose gradient (17). The control marker was prepared from uninfected Vero cells labeled and processed in the same way.*

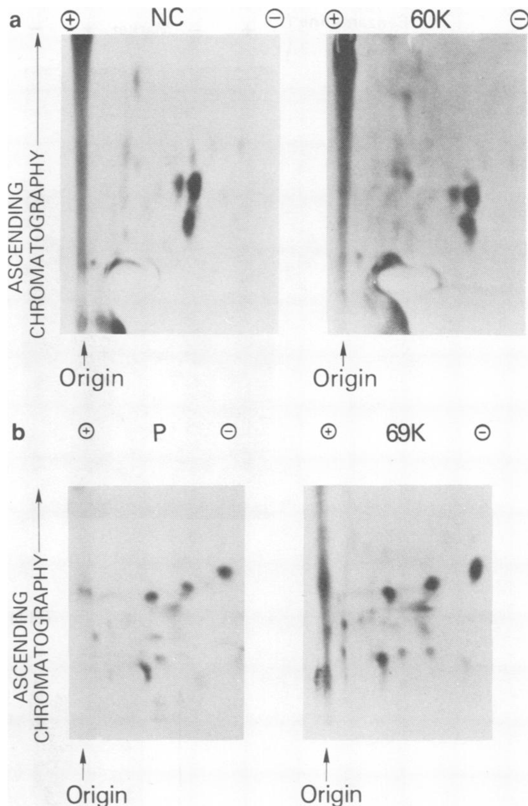

FIG. 2. *Two-dimensional thin-layer chromatograms of tryptic peptides of the synthetic 60K translation product and authentic viral NC polypeptide (a) and the 69K product and viral phosphoprotein (b). Authentic and synthetic viral polypeptide bands were electrophoretically eluted from preparative 10% polyacrylamide slab gels, oxidized with performic acid, and incubated with tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin (Worthington Biochemicals Corp.) before ascending chromatography in n-butanol-acetic acid-water-pyridine (150:30:120:120), followed by electrophoresis in the perpendicular direction for 30 min at 1,000 V and 8°C (3).*

lected cytoplasmic RNA labeled with [^{3}H]uridine in the presence of actinomycin D by density gradient ultracentrifugation through gradients of glycerol (15 to 30% in 0.01 M Tris, pH 7.4; SW60 rotor; 65,000 rpm; 5.5 h; 4°C) after denaturation in 50% formamide for 5 min at 65°C.

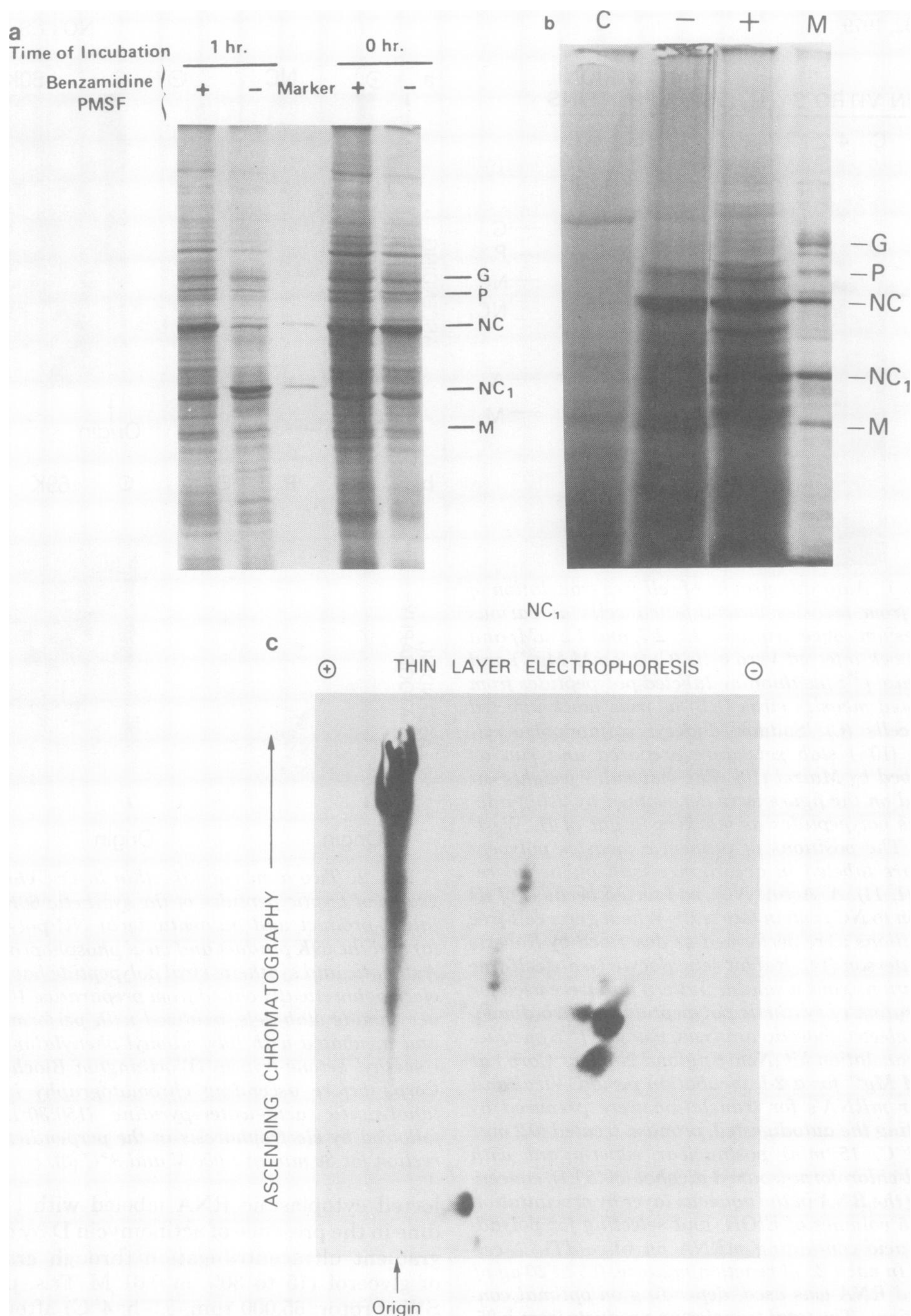

FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of virion polypeptides purified from cytoplasmic extracts of measles virus-infected cells with and without 50 μg of phenylmethylsulfonyl fluoride per ml and 1 mM benzamidine. Washed measles virus-infected cells were incubated for 1 h at room temperature with the indicated inhibitors and then prepared for gel electrophoresis as described in the legend to Fig. 1. Gels were prepared and run as described by Laemmli (8). Although the Laemmli and Maizel gel patterns appear different, the order of mobilities of the polypeptides is the same. The 48K size of NC_1 is based on electrophoresis in the Laemmli system. NC_1 runs as a higher-molecular-weight polypeptide in the Maizel system. The marker is Edmonston measles virus purified as described in the legend to Fig. 1. (b) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of translation assays incubated in the presence (+) and absence (−) of Vero cell cytoplasmic extract (postnuclear supernatant). Samples were incubated for 1 h at room temperature after the addition of cytoplasmic extract. Gels were prepared and run as described by Laemmli (8). C, Control marker; and M, Edmonston measles virus, prepared as described in the legend to Fig. 1. (c) Two-dimensional thin-layer chromatograms of tryptic peptides 48K (NC_1) prepared and run as described by Eron et al. (3).*

The majority of this RNA sedimented as a broad heterogeneous peak of 16 to 20S. Synthesis of polypeptides (69K, 60K, 44K, and 38K) was programmed only by those gradient fractions containing RNA in this size class (data not shown).

Virions isolated from measles virus-infected Vero cells, but not those released into the cell supernatant, showed an additional labeled polypeptide with an apparent molecular weight of 48K (Fig. 1, NC_1). This new band is more prominent in gels of virion polypeptides purified from cells infected with several subacute sclerosing panencephalitis isolates (J. Sprague, L. J. Eron, and P. Albrecht, unpublished observations). Previous studies with other paramyxoviruses (simian virus 5, Newcastle disease virus, and Sendai virus) have shown that the NC protein can exist in two forms: a large form with a molecular weight near 60K and a smaller form with a molecular weight near 45K. The smaller form arises from a highly specific proteolytic cleavage of the larger form after disruption of infected cells that have been dispersed with a protease (9, 11). More recent studies suggest the presence of several lower-molecular-weight forms of the measles NC polypeptide under certain conditions (18). To examine the possibility that the 48K measles virion polypeptide was a result of proteolytic cleavage of the NC protein by intracellular proteases, we prepared purified virus with the addition of protease inhibitors such as phenylmethylsulfonyl fluoride and benzamidine (Fig. 3a). The fact that the 48K protein is not present in samples treated with protease inhibitors suggests that this protein is a product of proteolytic cleavage. The 48K protein was not synthesized in the cell-free system. However, it could be generated by the addition of Vero cell extract to the translation assay, further substantiating a relationship to the 60K nucleocapsid (Fig. 3b). Finally, Fig. 3c shows that the tryptic digest fingerprint of 48K (NC_1) is similar to that of NC protein (Fig. 2a).

The data reported here indicate that measles virus mRNA isolated from infected cells can program in vitro translation systems to synthesize at least three polypeptides which are identified by tryptic digest fingerprints or two-dimensional electrophoresis as measles M, NC, and P proteins. In addition, a fourth measles virion polypeptide has been shown to be a proteolytic cleavage product of NC protein and can be produced in vitro by the addition of a Vero cell cytoplasmic extract to the translation system. The role of NC_1 in measles virus infection, and particularly in infections mediated by the variants associated with subacute sclerosing panencephalitis, is currently under investigation in this laboratory.

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