0022-538X/78/0025-0285$02.00/0
JOURNAL OF VIROLOGY, Jan. 1978, p. 285-297
Vol. 25, No. 1

Printed in U.S.A.

Intracellular Synthesis of Measles Virus-Specified Polypeptides

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Received for publication 21 July 1977

The intracellular synthesis of measles-specified polypeptides was examined by means of polyacrylamide gel electrophoresis of cell extracts. Since measles virus does not efficiently shut off host-cell protein synthesis, high multiplicities of infection were used to enable viral polypeptides to be detected against the high background of cellular protein synthesis. The cytoplasm of infected cells contained viral structural polypeptides with estimated molecular weights of 200,000, 80,000, 70,000, 60,000, 41,000, and 37,000. All of these structural polypeptides, with the exception of P1, the only virion glycoprotein (molecular weight ≃ 80,000), were also found in the nuclei. In addition, two nonstructural polypeptides with estimated molecular weights of 74,000 and 72,000 were also present in the cytoplasm of infected cells. The initial synthesis of the smaller, nonstructural polypeptide began later in infection than the structural polypeptides. Pulse-chase experiments failed to detect any precursor-product relationships. The intracellular glycosylation and phosphorylation of the viral polypeptides were found to be similar to those found in purified virions.

The involvement of measles virus in the slowly progressive neurological disease subacute sclerosing panencephalitis (SSPE) (2, 4, 10, 21) and its possible role in multiple sclerosis (5, 14, 15) have led to increased interest in the strategies of its replicative cycle. Preliminary characterization of virion polypeptides and RNA have been carried out, (3, 7, 8, 17, 37). However, there is no information available on the synthesis of measles polypeptides in infected cells. This is an area of particular interest because, unlike most conventional paramyxoviruses that replicate and produce inclusions almost exclusively in the cytoplasm, the host cell nucleus has been shown to have a possible role in measles virus replication, persistent measles infections, and SSPE (6, 31).

One of the major obstacles in the study of measles virus is the inability to obtain preparations of virus free of contaminating cellular proteins (17). This has made it difficult to establish whether virion-associated polypeptides are cell or viral coded. For example, as many as 10 to 20 minor bands, most of which are presumably cellular in origin, can be resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of purified measles virions.

The measles virion appears to contain seven viral-coded polypeptides (17, 37, and below) with estimated molecular weights of: L, 200,000; P1, 80,000; P2, 70,000; P3-NP, 60,000; P4, 55,000; P5, 41,000; and P6-M, 37,000. In addition, there is a major polypeptide (A, 43,000) that comigrates with cellular actin and probably is not virus specified (13, 36). Of the viral-coded polypeptides contained in the virion, one is a glycoprotein (P1) (17), and two are phosphoproteins (P2 and P3) (3). The most abundant polypeptide, P3, is the nucleocapsid protein (NP) (17, 37), whereas the smallest polypeptide, P6, is the nonglycosylated membrane protein, M (3, 17). Little is known about the largest polypeptide, L, or the polypeptides designated P4 and P5.

This report describes a characterization of the polypeptides synthesized in the cytoplasm and nucleus of cells infected with the Edmonston strain of measles virus.

MATERIALS AND METHODS

Cell lines. CV-1 (African green monkey kidney) cells were grown as monolayers in roller bottles in Richters improved minimal essential medium MEM containing zinc, insulin, and 10 mM HEPES buffer (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid [IMEMZO; International Biological Laboratories]) supplemented with 10% fetal calf serum. Vero and HeLa cells were grown as monolayers in tissue culture flasks in the same medium.

Virus. The Edmonston strain of measles virus was used for all experiments. Viral stocks were prepared by infecting subconfluent monolayers of CV-1 cells with a multiplicity of infection (MOI) of 0.01 to 0.05 PFU/cell in 0.5 ml of medium. After 2 h of adsorption

at 33°C, the monolayers were fed with 20 ml of IMEMZO per 150-cm^2 flask. Passages were made at 33°C. When cytopathic effect (CPE) included approximately 80% of the monolayer, the infected cultures were harvested by freezing and thawing at −70°C. This procedure gave viral yields as high as 3×10^7 PFU/ml. Stocks were stored at −70°C. Virus was plaque purified twice and passaged four to five times as described before used.

Isotopic labeling and purification of virus. CV-1 monolayers in roller bottles (10^8 cells per bottle) were infected with virus in 5 ml of medium at an MOI of 0.01. After 2 h of adsorption at 37°C, the inoculum was removed, and each roller bottle was fed with 50 ml of IMEMZO. Twenty hours postinfection, the medium was removed and relaced with 10 ml of methionine-free Eagle MEM supplemented with 10% fetal calf serum and containing 0.5 mCi of [^{35}S]methionine (Amersham/Searle; specific activity, >400 mCi/mmol). The medium was changed at 12-h intervals until 20 to 50% of the cells were no longer attached to the glass. At each harvest the medium was clarified of cell debris (1,000 × *g* for 30 min) and stored at −70°C. After the final harvest, the clarified medium was thawed and pooled. Virus was pelleted at 25,000 rpm in an SW27 rotor for 2 h. The pellet was suspended in 1 to 2 ml of 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) and layered over a 15 to 50% (wt/vol) linear potassium tartrate gradient. The gradient was centrifuged for 4 h at 25,000 rpm in an SW27.1 rotor. The visible viral band (density, 1.23 g/cm^3) was collected, diluted with 1× SSC, pelleted, and rebanded as before. The viral band from the second gradient was collected, pelleted, and suspended in sample gel buffer (12) in preparation for SDS-PAGE.

Isotopic labeling of intracellular viral proteins. Confluent monolayers of CV-1, Vero, or HeLa cells in Falcon multicluster wells (Falcon 3008) (10^5 cells per well) were infected with virus at an MOI of 50, unless otherwise specified. After 2 h of adsorption at 33°C the infected monolayers were fed with 1 ml of IMEMZO. Incubation was carried out at 33°C in a 5% CO_2 atmosphere. [^{35}S]methionine incorporation was carried out in 0.5 ml of methionine-free Eagle MEM containing 100 μCi of [^{35}S]methionine (specific activity, >400 mCi/mmol). Before [^{35}S]methionine labeling, the monolayers were washed and preincubated for 1 h in methionine-free medium. ^{32}P-labeling was carried out in IMEMZO containing 250 μCi of inorganic ^{32}P per ml (New England Nuclear). [^{14}C]glucosamine labeling was done in glucose-free MEM containing 25 μCi of [^{14}C]glucosamine per 0.5 ml of medium. When [^{35}S]methionine pulse-chase experiments were performed, an effective chase was achieved by removing the labeled medium, washing the monolayer two times with IMEMZO, and feeding the monolayer with IMEMZO containing the normal amount of unlabeled methionine. No further incorporation could be detected during this chase procedure.

Termination of pulse and pulse-chase procedures was accomplished by rinsing the monolayers two times with ice-cold TMN (0.01 M Tris–0.0015 M $MgCl_2$–0.14 M NaCl [pH 7.2]). The cells were then collected with a glass rod and suspended in 1 ml of TMN.

Preparation of cell extracts. Labeled-cell suspensions (10^5 cells per ml of TMN) were made 0.5% with respect to Nonidet P-40 (NP-40, Particle Data Laboratories, Ltd.) and incubated on ice for 30 min. The samples were centrifuged at 1,000 × *g* for 1 min. The supernatant represents the cytoplasmic fraction and the pellet, the nuclear fraction (22). The nuclei were suspended in 1 ml of TMN, and 10 volumes of −20°C acetone was added to both fractions. The samples were incubated overnight at −20°C. The acetone-precipitated polypeptides were pelleted at 1,500 × *g* for 2 h. The acetone was poured off, and the pellets were allowed to air dry for 24 h at room temperature. Each of the nuclear and cytoplasmic pellets was then suspended in 0.3 ml of gel sample buffer (12), and 10-μl fractions were subjected to electrophoresis. To examine the degree of nuclear purification, NP-40-prepared nuclei were treated with a combination of 0.67% Tween 40 and 0.33% sodium deoxycholate for 10 min and then pelleted as above (22). No difference in protein composition was detected between infected nuclei prepared by these two methods.

PAGE. SDS-PAGE of proteins was carried out by the method of Laemmli (12). Electrophoresis was carried out in a 10% polyacrylamide-slab gel at a constant current of 45 mA for 2 to 3 h. Gels were fixed in 50% methanol–7% acetic acid for 1 h, dried under vacuum, and exposed to autoradiography with Kodak NS2T Safety Screen X-ray film. Molecular weight estimates were made by comparison to reovirus proteins of known molecular weight.

RESULTS

Virion polypeptides. Measles virus, labeled with [^{35}S]methionine, was purified from infected CV-1 cells. Analysis of the purified virion polypeptides by SDS-PAGE revealed six major and three or more minor polypeptides (Fig. 1). The estimated molecular weights of these polypeptides are L, 200,000; P1, 80,000; P2, 70,000; P3-NP, 60,000; P4, 55,000; α, 46,000; A (presumably cellular actin), 43,000; P5, 41,000; and P6-M, 37,000 as determined by comparison to reovirus polypeptides of known molecular weights. This polypeptide pattern is similar to results reported for measles by other groups (17, 37).

Since measles virus preparations free of significant quantities of cellular protein have not been obtained (17), it is difficult to establish whether minor components present in the virion are viral or host coded. Several minor bands with estimated molecular weights ranging from 56,000 to 44,000 (between the NP and A bands) were detected in our viral preparations. The amounts of these minor components varied greatly in different viral preparations. Of these minor components we have been able to detect only one in extracts of infected cells. To distinguish it from the others we have labeled this minor band α. Although we have not yet detected the polypeptide designated P4 in extracts of infected cells (possibly because of excessive

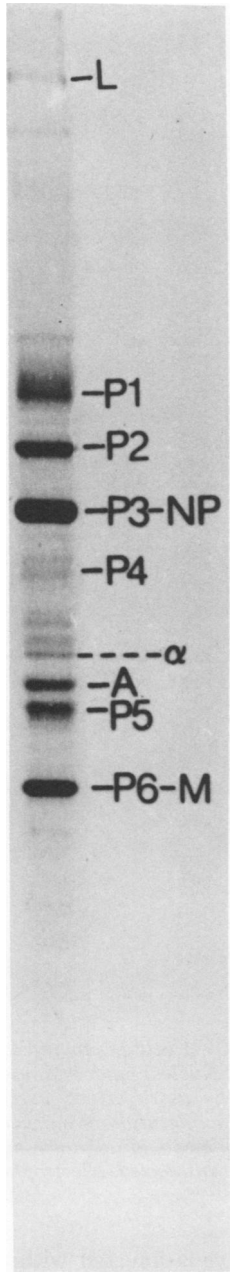

FIG. 1. *PAGE of [^{35}S]methionine-labeled polypeptides from purified measles virions. Virus was grown and labeled in CV-1 cells. Virus was purified and processed for electrophoresis as described in Materials and Methods. Migration is from top to bottom. NP, Nucleocapsid protein; α, minor component; A, cellular actin; M, nonglycosylated membrane protein. Electrophoresis was for 2 h.*

cellular background), we have found that this band is occasionally fairly prominent in purified virions. This variability in the amount of P4 present in different viral preparations agrees with the work reported by Mountcastle and Choppin (17).

In addition to the minor bands between NP and A, several minor bands migrating between L and P1 are also present in our viral preparations. Except for the band closest to P1 (estimated molecular weight, ~90,000), each of these minor bands comigrates with a cellular band and, therefore, is presumed to be cellular in origin. The 90,000-molecular-weight band has occasionally been seen in extracts of infected cells (Fig. 2, top arrow); however, its significance is unknown.

Effect of MOI on detection of viral polypeptides in extracts of infected cells. (i) Cytoplasmic polypeptides. The analysis of intracellular viral polypeptides is complicated by the fact that measles virus does not efficiently shut off host cell protein synthesis. We have found that viral polypeptides are most readily detected when cells are infected at a high-input MOI. Under these conditions, the relatively large quantities of viral polypeptides are easily detected over the cellular background. At a low MOI (<1 PFU/cell) viral CPE generally appeared later than 36 h postinfection and was characterized by widely spaced plaquelike cell destruction. When intracellular viral polypeptides were examined under these conditions, the cellular polypeptides were very prominent, and, thus, even very late postinfection, viral polypeptides were not readily detected over the cellular background.

To examine in detail the effect of MOI on the synthesis of viral polypeptides, CV-1 cells were infected with wild-type measles at MOI values of 0.1 to 50. When the higher MOI infections showed virtually 100% CPE, the monolayers were pulse-labeled with [^{35}S]methionine. At low MOI values, viral polypeptides were difficult to detect above the cell background (Fig. 2). However, at MOIs of 10 and 50, polypeptides L, P1, P2, NP, P5, and M could be easily seen in cytoplasmic extracts. Two additional cytoplasmic, virus-related bands located between P1 and P2 were also present. Under appropriate conditions (high MOI and late in infection), these two bands are reproducibly detected. Since we have never detected these bands in purified virus, they have been designated nonstructural proteins (NS) 1 and 2. The cytoplasmic polypeptides synthesized after infection at a high MOI contained two additional minor bands (Fig. 2A, unlabeled arrows) not present in uninfected samples. These bands are not usually detected, and their significance is not known.

(ii) Nuclear polypeptides. Since the precise role of the nuclear phase of measles replication

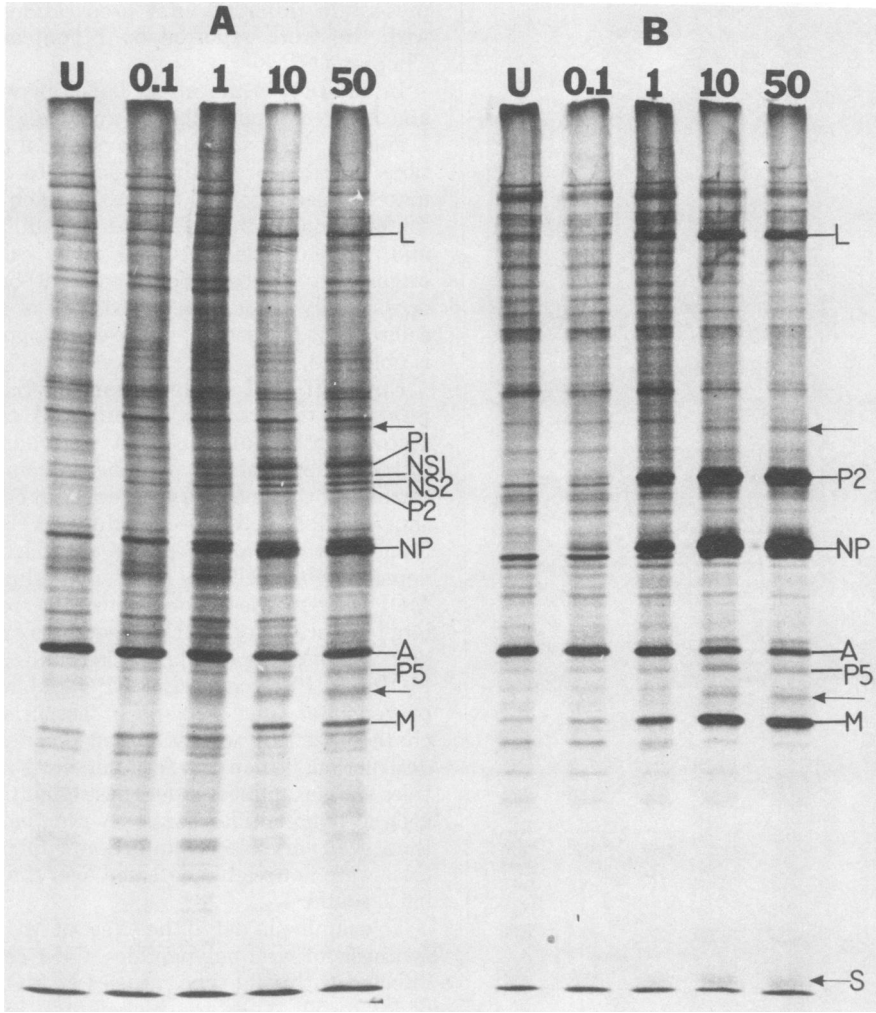

FIG. 2. *Effect of MOI on measles-specified polypeptide synthesis in CV-1 cells. Cells infected at various MOIs were labeled with [^{35}S]methionine for 2 h at 24 h postinfection. Nuclear and cytoplasmic fractions were prepared and processed for electrophoresis and autoradiography as described in Materials and Methods. The positions of the viral polypeptides were determined by the migration of marker polypeptides in another lane on the slab gel. Unlabeled arrows indicate minor components of unknown significance. A, Cytoplasmic fractions; B, nuclear fractions. Numbers indicate MOI; U, uninfected. Electrophoresis was for 2 h.*

is not understood (16, 31), we examined infected-cell nuclei for the presence of measles virus polypeptides. Nuclear preparations from cells infected at a high MOI revealed the following polypeptides: L, P2, NP, P5, M, and the two minor undesignated bands (Fig. 2B). In addition, a low-molecular-weight band (less than 20,000), designated S for small, was visible. Occasionally, S was also present in infected cytoplasmic samples.

P1, NS1, and NS2 were absent from the nuclear extracts. This was demonstrated most clearly when portions of cytoplasmic and nuclear extracts from cells infected with a high MOI (Fig. 2A and B) were placed on adjacent gel tracts and subjected to SDS-PAGE for longer periods of time (Fig. 3). P1, NS1, and NS2 were clearly present only in the cytoplasm. NP and M were present in large amounts in both cell fractions. On the other hand, P2 seemed to be concentrated mainly in the nuclear fraction (the distribution of P2 changes with time; see kinetic experiments).

A comparison of the electrophoretic pattern of NP in cytoplasmic and nuclear extracts revealed that NP derived from the cytoplasm mi-

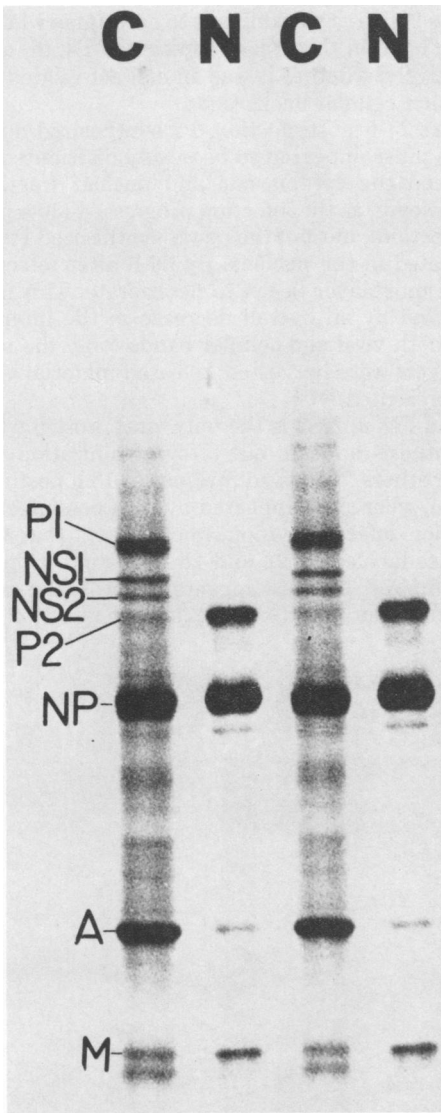

FIG. 3. *Comparison of cytoplasmic and nuclear viral polypeptides. Portions of the cytoplasmic and nuclear samples in Fig. 2 infected at an MOI of 50 were subjected to electrophoresis for 2.5 instead of 2 h. This increases the separation of bands (particularly the P1 to P2 region), but results in decreased resolution of other viral and cellular bands (i.e., L and P5 are no longer resolved, and the cell background in the nuclear samples is greatly diminished compared with Fig. 2). C, Infected cytoplasm; N, infected nuclei (duplicate samples of C and N are displayed).*

grates slightly more rapidly. Although this difference in migration was often seen (see Fig. 4 and 6), its significance is not clear.

To ensure that nuclear fractions were not contaminated by cytoplasmic material, two control tests were performed. First, NP-40-prepared nuclei were further purified by a double-detergent treatment that completely removed the outer nuclear membrane (22). Nuclei from both infected and uninfected cells prepared in this manner were subjected to SDS-PAGE. The polypeptide patterns obtained were indistinguishable from those of NP-40-purified nuclei (data not shown). The second test involved a reconstruction experiment (Table 1). [^{35}S]methionine-labeled cytoplasm and unlabeled nuclei were mixed, and the nuclei were reisolated by centrifugation. Under these conditions, less than 1% of the cytoplasmic label pelleted with the nuclei. The results of these experiments indicate that there was no significant contamination of NP-40-purified nuclei.

Effect of MOI on viral growth. To ensure that the high MOI needed for the detection of intracellular viral polypeptides did not result in abortive or abnormal viral growth, the yield of virus was examined under conditions similar to those used for polypeptide labeling. In the first 30 h postinfection the rate of viral growth was similar at all of the MOI values examined between 0.1 and 50 (data not shown). In addition, all input MOIs resulted in approximately the same maximal virus yields. The only difference noted was that, at a high MOI, the peak yield was attained sooner (36 h postinfection at an MOI of 50 compared with 72 h postinfection at an MOI of 0.1). Thus, the conditions used here for detection of intracellular viral polypeptides allowed relatively normal viral growth.

TABLE 1. *Analysis of possible contamination of nuclear fraction by radiolabeled cytoplasm*[a]

Fraction	Total cpm	Recovery (%)
Total cell	1.46×10^6	100
Cytoplasm	1.23×10^6	84.2
Nuclei	2.18×10^5	14.9
Reconstruction		
Labeled cytoplasm and unlabeled nuclei	9.22×10^5	100
Cytoplasm	9.30×10^5	100
Nuclei	7.65×10^3	0.8

[a] Two monolayers of CV-1 cells were infected with measles virus at an MOI of 50. Thirty hours postinfection (at 33°C) one monolayer was pulse-labeled with [^{35}S]methionine for 2 h as described in Materials and Methods. The second monolayer remained unlabeled. Cytoplasmic and nuclear fractions were prepared by NP-40 treatment as described in Materials and Methods. Labeled cytoplasm and unlabeled nuclei were mixed and incubated at 4°C for 30 min. The nuclei were reisolated by centrifugation. At each stage, portions were counted in an aqueous scintillation fluid, and the total counts per minute present in the original samples were calculated.

Kinetics of appearance of viral polypeptides. Since viral polypeptides could be easily distinguished from cellular polypeptides at an MOI of 50, the time of first appearance and maximal synthesis of intracellular virus-specified polypeptides was determined under these conditions (Fig. 4). At 12 to 18 h postinfection, L, P1, NS1, P2, NP, and M began to appear. By 24 h postinfection, all the viral polypeptides (except P5, which is not resolved by this gel, and P4, which may be masked by cellular polypeptides) were clearly visible. The same general pattern of viral polypeptides was also seen when viral growth was carried out at 38 instead of 33°C. However, at 38°C, viral polypeptides appeared sooner and could all be readily detected by 14 h after infection (data not shown).

By 24 h postinfection at 33°C, a band in the P4 region (just below NP) appeared to become prominent (Fig. 4, 24 h, cytoplasm). However, at later times postinfection it was no longer as intense. Since uninfected cytoplasmic extracts often contain a prominent band in this region (see Fig. 6), it was difficult to determine whether the band in this track represented P4, or, alternatively, whether it was an artifact related to a higher cellular background.

At 24 h postinfection, P2 synthesized during the pulse appeared to be evenly distributed between the cytoplasmic and nuclear fractions. However, as the infection progressed (30 h postinfection), most of the newly synthesized P2 was located in the nucleus. By 36 h after infection, the monolayer began to deteriorate. This is reflected by an overall decrease in the intensity of both viral and cellular bands (only the monolayers were harvested; released material is not represented).

In Fig. 4, NS2 is the only viral protein whose synthesis appears out of synchronization with the others. NS2 is not present at 18 h postinfection, whereas it appeared by 24 h postinfection. When infected cytoplasmic extracts that were pulse-labeled at 22 and 28 h postinfection are compared, the late appearance of NS2 is more easily seen (Fig. 5). At 22 h after infection, P1,

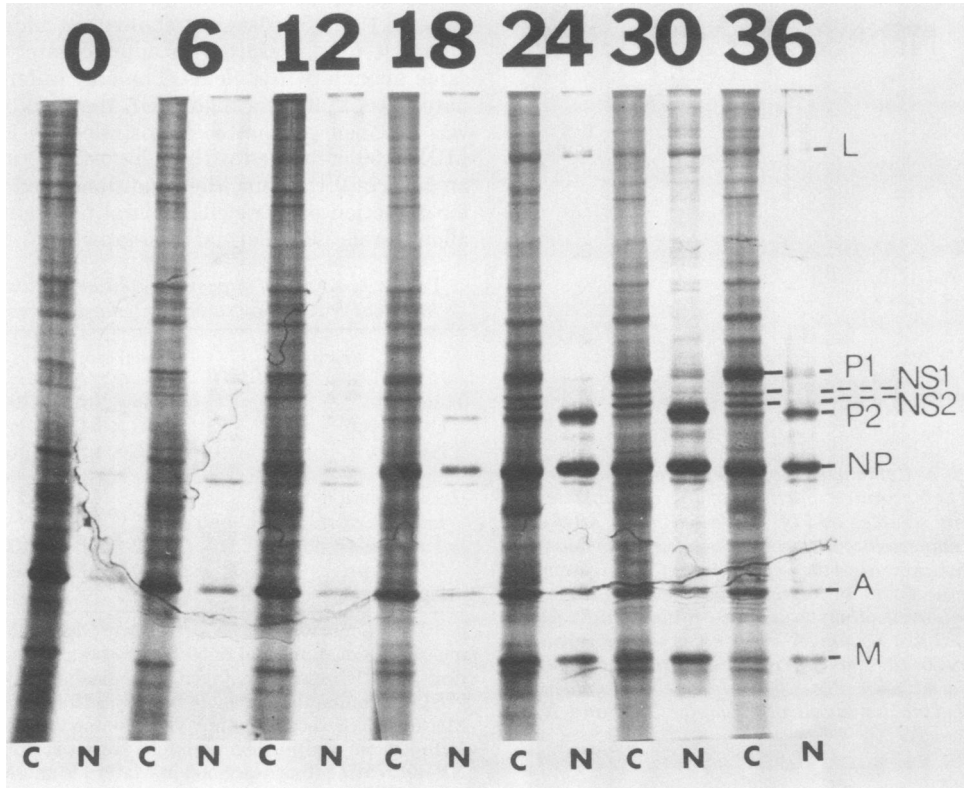

FIG. 4. *Synthesis of polypeptides in measles-infected cells; cytoplasmic and nuclear fractions at various times after infection. Uninfected and infected CV-1 cells were labeled with [^{35}S]methionine for 2 h at various times postinfection and processed for electrophoresis and autoradiography. Numbers indicate hours after infection. C, Cytoplasmic fractions; N, nuclear fractions. Electrophoresis was for 2.5 h.*

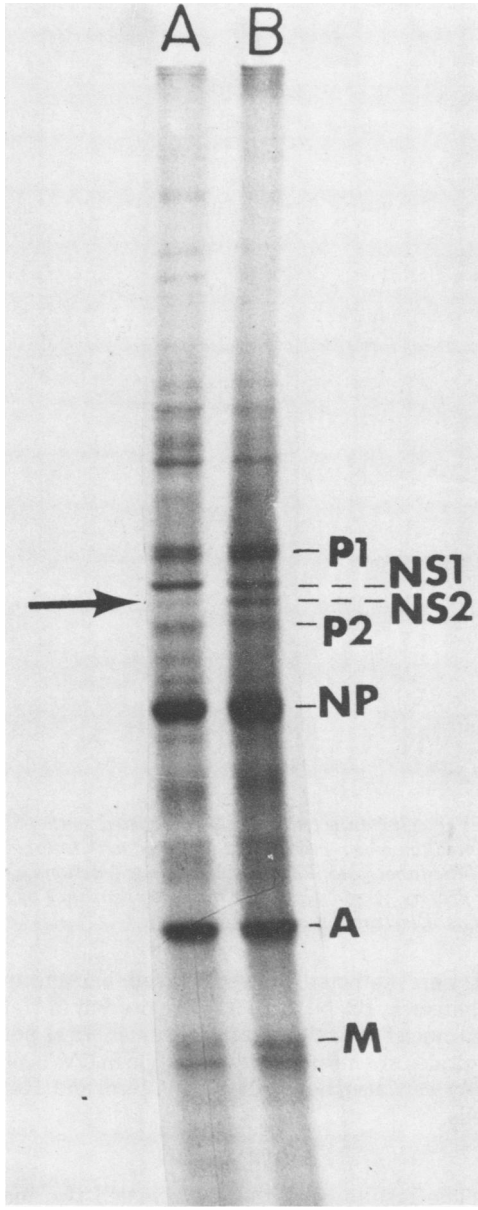

FIG. 5. *Late appearance of a nonstructural protein in cytoplasmic extracts of measles-infected cells. Infected CV-1 cells were labeled with [^{35}S]methionine for 2 h at either 22 or 28 h postinfection. Cytoplasmic extracts were prepared and processed for electrophoresis and autoradiography. Arrow indicates missing NS2. A, 22 h postinfection; B, 28 h postinfection. Electrophoresis was for 2.75 h.*

NS1, and P2 were present in the cytoplasm, whereas NS2 was absent (arrow). However, by 28 h postinfection, NS2 had become a major cytoplasmic component.

Pulse-chase experiments. Pulse-chase experiments were undertaken to look for possible precursor-product relationships. At 22 h postinfection, infected monolayers were pulse-labeled for 20 min and chases were carried out for up to 8 h (Fig. 6). (Attempts to achieve significant labeling of viral polypeptides with shorter pulses were unsuccessful.) At the time of pulse-labeling, all the viral proteins except NS2 were being synthesized (Fig. 6, lane 0). As the cells were chased, no new bands became visible (20 min to 8 h). In particular, NS2 does not appear during a chase of up to 8 h. This time interval (22 to 30 h postinfection) covers the period during which NS2 could be pulse-labeled. The failure of NS2 to appear during a chase suggests that it is not a breakdown or cleavage product of a precursor protein. Rather, NS2 appears to be a unique polypeptide whose synthesis is initiated later than the other viral proteins.

In chases of up to 2 h nothing could be detected to differentiate the pulse-chase from a simple pulse. By 4 to 8 h of chase, however, P1 begins to disappear from the cytoplasm (Fig. 6, arrows). This decrease in P1 is not accompanied by any visible increase in any other viral bands and may represent cellular loss due to budding out of mature virus.

As is usually the case, P4 and P5 could not be detected in these cellular extracts. Thus, the likelihood that they are cleavage products of other polypeptides cannot be confirmed. Pulse-chase experiments have, thus far, been unsuccessful in detecting precursor-product relationships among any viral polypeptides.

A comparison of the electrophoretic pattern of P2 in cytoplasmic and nuclear extracts (Fig. 6) revealed that P2 derived from the cytoplasm migrates slightly faster than P2 derived from the nucleus. Although this difference in migration is occasionally seen (Fig. 4), its significance is unclear.

Protein modifications. (i) Phosphorylation. Purified measles virions contain two phosphorylated proteins (P2 and NP) (3). We, therefore, examined infected-cell extracts to see if viral-specific phosphoproteins could be detected (Fig. 7). The cytoplasm of infected cells contained four phosphorylated bands (arrows, track C) not found in control cells. Two of the bands correspond to P2 and NP. The third was in the vicinity of P5. The much fainter fourth band was located between NP and A in the general vicinity of α and the other minor bands found in purified virus. Because of the high sensitivity of NP and possibly P2 to proteolytic cleavage by both cellular and extracellular protease (17, 38), we suspect that the latter two ^{32}P-labeled bands, which are not present in purified virions, are breakdown products of NP or P2. In infected

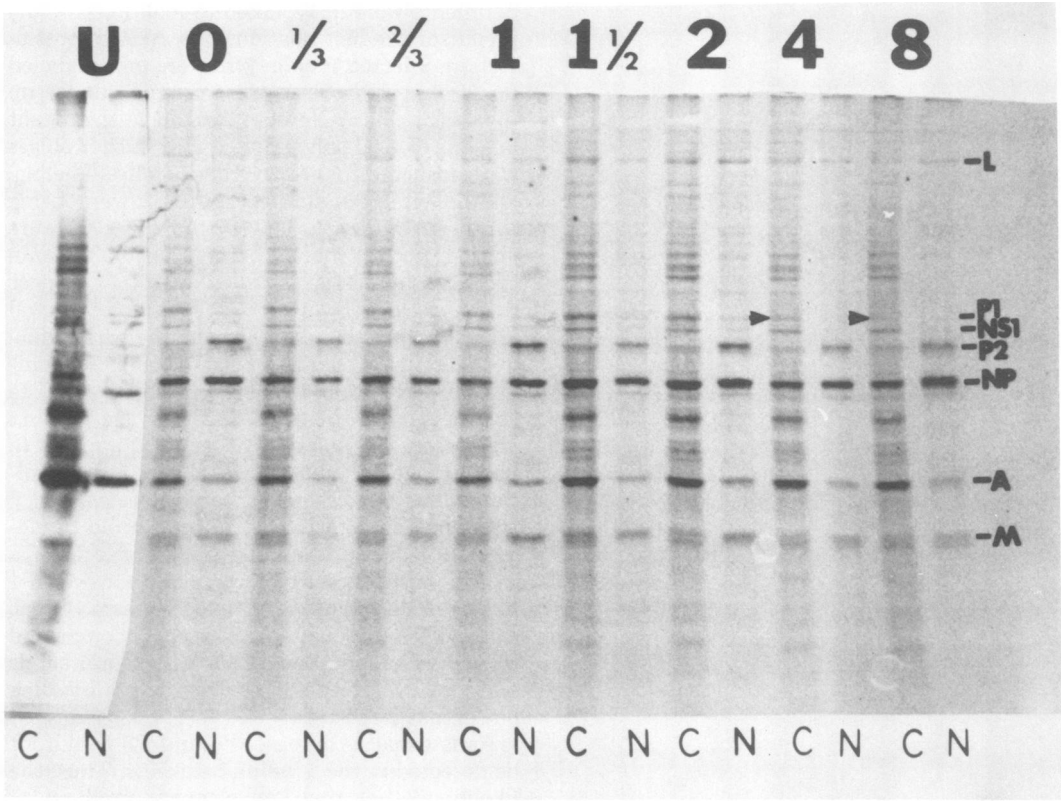

FIG. 6. *Pulse-chase experiments in measles-infected CV-1 cells. Infected cells were labeled with [^{35}S]-methionine for 20 min at 20 h after infection. The labeling medium was removed and replaced with unlabeled medium. At the indicated times (in hours) after removal of the label, cytoplasmic and nuclear fractions were prepared and processed for electrophoresis and autoradiography. Arrows indicate the disappearance of P1. U, Uninfected; C, cytoplasmic fractions; N, nuclear fractions. Electrophoresis was for 2.25 h.*

nuclei only two proteins, P2 and NP, are phosphorylated (Fig. 7, track D, arrows).

(ii) Glycosylation. [^{14}C]glucosamine labeling in infected cells was examined to determine the intracellular pattern of glycosylation of viral polypeptides (Fig. 8). As in purified measles virions (17), P1 is the only viral polypeptide labeled by [^{14}C]glucosamine. P1 thus appears to be a glycoprotein in both purified virus and cell extracts.

HeLa and Vero cell extracts. The extent of reproducibility of viral polypeptide patterns was examined in Vero (Fig. 9) and HeLa (Fig. 10) cells. In Vero cell cytoplasms, P1, P2, NP, α, P5, and M could be detected (Fig. 9A). P1, NP, and M were present in sufficient quantity to be readily observed. P2 and α were somewhat masked by the cellular background, whereas P5 was barely detectable. In Vero cell nuclei, L, P2, NP, α, and M were clearly visible (Fig. 9B). In addition, a small amount of S was also present. In HeLa cell cytoplasms, only P1, NP, and M were detected above the cell background, whereas L, P2, NP, and M were present in HeLa cell nuclei (Fig. 10). Thus, although viral polypeptides are most easily detectable in CV-1 cells, they can also be observed in Vero and HeLa cells.

DISCUSSION

The results of this study revealed two main facts: (i) measles-specified polypeptides can be readily analyzed by SDS-PAGE of cell extracts, and (ii) the nuclei of cells infected with measles contain all of the measles structural proteins that are found in the cytoplasm, with the exception of the surface glycoprotein P1.

The major difficulty encountered in detection of intracellular measles polypeptide synthesis was finding that measles virus does not shut off host cell protein synthesis. High MOI was, thus, used to increase the quantity of viral proteins synthesized. Under these conditions, the viral polypeptides can be readily detected in infected-

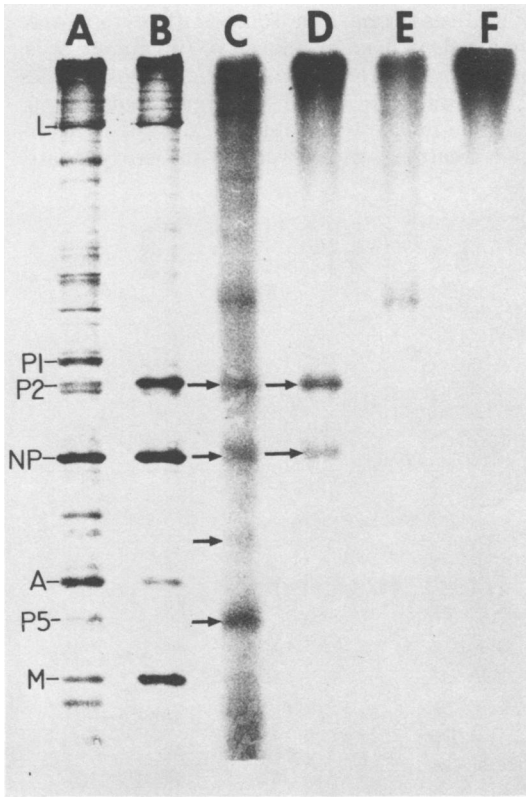

FIG. 7. *Phosphoprotein synthesis in measles virus-infected CV-1 cells. Uninfected and infected cells were labeled with either [^{32}P]- or [^{35}S]methionine for 2 h at 24 h postinfection. Cytoplasmic and nuclear extracts were prepared and processed for electrophoresis and autoradiography. A, [^{35}S]methionine-labeled infected cytoplasm; B, [^{35}S]methionine-labeled infected nuclei; C, ^{32}P-labeled infected cytoplasm; D, ^{32}P-labeled infected nuclei; E, ^{32}P-labeled uninfected cytoplasm; F, ^{32}P-labeled uninfected nuclei. Arrows indicate ^{32}P-labeled bands found in extracts of infected and not in uninfected cells. Electrophoresis was for 2 h.*

cell extracts even in the presence of a high background of cellular polypeptides.

Involvement of the nucleus in measles infection is well documented, although its role is unclear. The yield of measles virus from infected enucleated cells is approximately 100-fold lower than the yield from nucleate cells (6). Immunofluorescent studies indicate that measles antigen is present in the nuclei of cells both acutely and persistently infected with measles virus (24, 26). Electron and light microscopy have revealed nucleocapsid-like viral inclusions in the nuclei of a variety of cells infected with measles virus (1, 11, 19, 25, 33, 34). In addition, brain cells from patients with SSPE contain viral inclusions almost exclusively in the nucleus. These inclusions resemble those seen in both the cytoplasm and the nucleus of measles-infected cells (2, 20, 23, 31, 32, 35). The nucleus is, thus, involved in measles infection and may well play a critical role in the determination of persistent infections and SSPE.

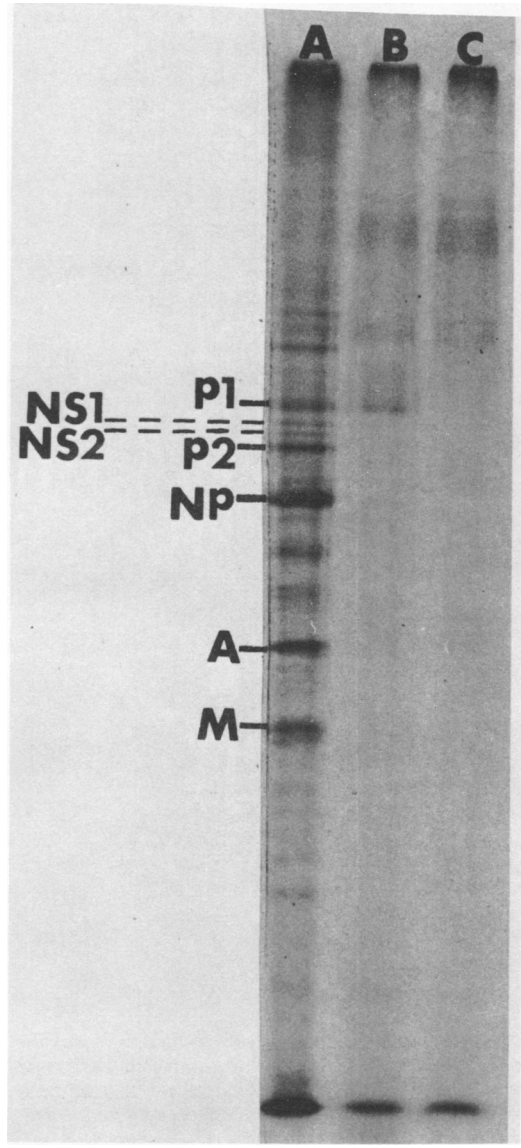

FIG. 8. *Glycoprotein synthesis in measles virus-infected CV-1 cells. Uninfected and infected cells were labeled with [^{14}C]glucosamine or [^{35}S]methionine for 8 h at 20 h postinfection. Cytoplasmic extracts were processed for electrophoresis and autoradiography. A, Infected, [^{35}S]methionine labeled; B, infected, [^{14}C]glucosamine labeled; C, uninfected, [^{14}C]glucosamine labeled. Electrophoresis was for 2.5 h.*

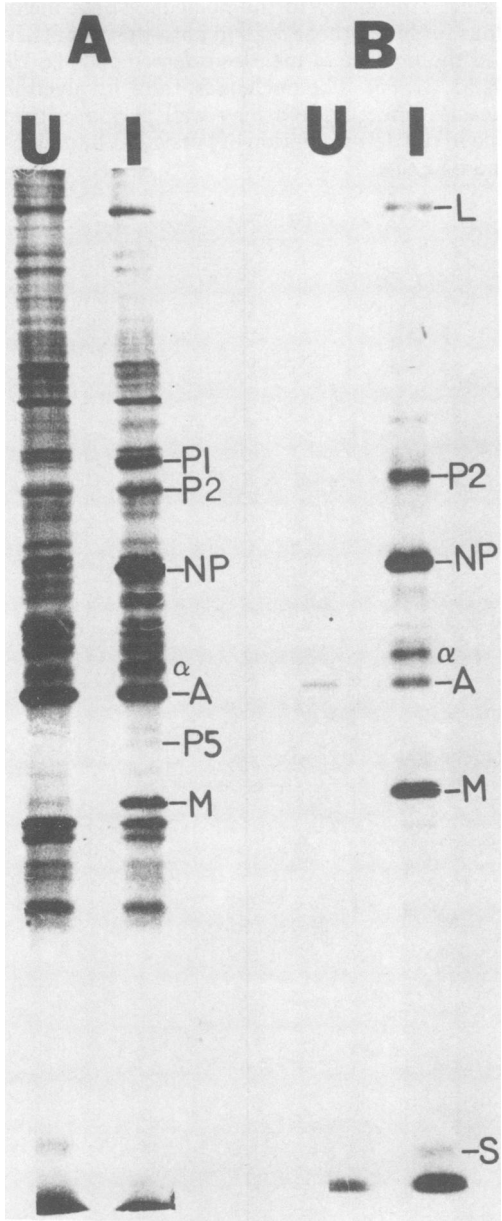

FIG. 9. *Polypeptide synthesis in measles virus-infected Vero cells. Infected and uninfected Vero cells were labeled with [^{35}S]methionine for 2 h at 24 h postinfection. Cell extracts were processed for electrophoresis and autoradiography. A, Cytoplasm; B, nuclei; U, uninfected; I, infected. Electrophoresis was for 2 h.*

To gain more information regarding the role of the nucleus in measles-infected cells, we routinely analyzed nuclear as well as cytoplasmic polypeptides. Nuclei were routinely prepared by NP-40 treatment. Contamination of these nuclei could arise from four sources: (i) cytoplasmic material, (ii) cell membranes, (iii) intact cells, and (iv) virions or subviral particles. Possibilities (i) and (ii) were tested and eliminated. NP-40-purified nuclei were subjected to further detergent treatment with Tween 40 and deoxychoate.

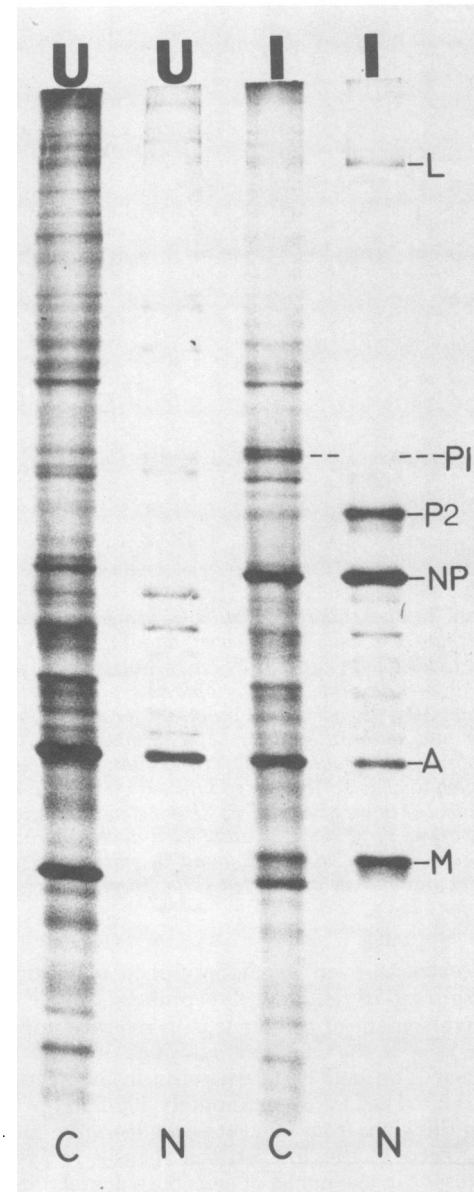

FIG. 10. *Polypeptide synthesis in measles virus-infected HeLa cells. Infected and uninfected HeLa cells were labeled with [^{35}S]methionine for 2 h at 24 h postinfection. Cell extracts were processed for electrophoresis and autoradiography. U, Uninfected; I, infected; C, cytoplasmic extracts; N, nuclear extracts. Electrophoresis was for 2 h.*

This procedure completely removes the outer nuclear membrane, resulting in highly purified nuclei free of both cytoplasmic material and cell membranes (22). SDS-PAGE analysis of these rigorously purified nuclei yields a viral polypeptide pattern identical to that obtained with NP-40-purified nuclei (data not shown). In addition, reconstruction experiments indicate that there is less than 1% contamination of nuclei by cytoplasmic polypeptides. The third possibility (intact cells) could not contribute significantly. Light microscopy revealed >99% disrupted cells after NP-40 treatment. The fourth possibility (viral particles) is very unlikely. In these preparations nuclei are pelleted at very low speeds for very short times (1,000 rpm for 1 min). Virions and subviral particles do not pellet under these conditions. In addition, the absence of P1 excludes complete virus. We are, therefore, quite confident that these nuclear preparations contain only nuclei.

The host-cell nucleus contains large quantities of the viral polypeptides L, P2, NP, and M. The presence of nucleocapsid-like structures in the nucleus makes detection in the nucleus of nucleocapsid-related proteins such as NP, P2, and L (3, 17) an expected finding. Absence in the nucleus of the glycoprotein, P1, is not surprising since this polypeptide would be expected to accumulate at the cytoplasmic membrane. On the other hand, the presence in the nucleus of the nonglycosylated membrane protein, M, was unexpected. This very hydrophobic protein, like P1, might be expected to accumulate at the cell membrane. The presence of large amounts of M in the nucleus is of interest but currently is not well understood. The absence of NS1 and NS2 from the nucleus indicates that the function (if it exists) of these nonstructural proteins is associated with the cytoplasm.

In addition to the absence of some viral polypeptides from the nucleus, three other differences exist between the viral polypeptides found in the nucleus and the cytoplasm. (i) The distribution of P2 between the cytoplasm and the nucleus, as detected by pulse-labeling, changes with time. Early in infection P2 is present in similar quantities in both cell fractions. However, later in infection, newly synthesized P2 is found mostly in the nucleus. (ii) The nucleocapsid protein, NP, isolated from nuclei usually migrates more slowly on SDS-PAGE than NP isolated from cytoplasm. (iii) P2 isolated from nuclei sometimes migrates more slowly than P2 isolated from the cytoplasm. The significance of these differences between the viral proteins in the cytoplasm and the nucleus and the nature of the role of the nucleus in measles and SSPE has not yet been determined.

The time course of polypeptide synthesis revealed that all of the viral-related polypeptides, except NS2, first appeared at approximately the same time postinfection. In addition, no difference could be detected between the initial time of appearance of the proteins in the nucleus and in the cytoplasm. This finding is in contrast to electron microscopy which reveals that viral inclusions appear in the cytoplasm before they appear in the nucleus (19). Pulse-chase experiments failed to reveal large precursor molecules involved in measles virus polypeptide synthesis. However, as seen in Newcastle disease virus (9, 18), these results do not eliminate the possibility of precursor polypeptides. Pulse-chase experiments also did not reveal transport of polypeptides from cytoplasm to nucleus, presumably because transport occurs too rapidly to be detected under the experimental conditions used here.

Two polypeptides, designated NS1 and NS2, are found in the cytoplasm of infected CV-1 cells late in the infection but not in purified virus. In addition, NS1 and NS2 were not detected in infected Vero and HeLa cell extracts. This suggests either stimulation of host cell proteins by viral infection or differential host cell modification, or breakdown, of viral polypeptides. It is also possible that NS1 and N2 have not yet been detected in these less permissive cell lines simply because they are produced in smaller quantities.

The measles virus genome consists of one nonsegmented, single-stranded RNA molecule with a molecular weight of approximately 6.0×10^6 to 6.2×10^6 (19, 30), or a maximum coding capacity of 6.2×10^5 daltons of protein. The total molecular weight of all the intracellular viral-related proteins presented here (L, P1, NS1, NS2, P2, NP, α, P5, and S = 7×10^5) exceeds this theoretical limit by approximately 80,000 daltons. Thus, if all of these polypeptides are viral coded, it is unlikely that they are all unique species.

Several virus-specific bands exhibited variability in their quantities from preparation to preparation. In purified virions some of the minor bands, including P4 shown in Fig. 1, are sometimes more abundant. In addition, P5 is occasionally present only in very minor amounts. In infected-cell extracts the α and P5 bands range from being undetectable to being as intense as NP or M. Although this variability may simply be due to instability of these polypeptides, it is possible that they are breakdown products or modifications of other polypeptides. If α, P5, and S are assumed to be secondary

gene products, then the viral genome would contain enough information to code for all of the remaining intracellular viral polypeptides presented in this paper. This excludes P4, which we have not detected in cell extracts and, thus, may be a cellular contaminant that appears in purified virions. In Newcastle disease virus, several minor bands with molecular weights similar to α and P5 were shown by tryptic peptide analysis to be related to NP (9). In Sendai, simian virus 5, and Newcastle disease virus, (27, 28, 29; A. Schied and P. W. Choppin, Virology, in press) a band with an electrophoretic mobility similar to S has been shown to be a cleavage product of another viral polypeptide. The coding capacity considerations discussed above suggest that these relationships may also be the case in measles.

We have presented a method for easily examining measles-specified polypeptide synthesis in both the cytoplasm and nucleus of infected cells. This should prove to be an excellent handle for the study of SSPE and persistently infected cells.

ACKNOWLEDGMENTS

The expert technical assistance of K. B. Byers is gratefully acknowledged.

This work was supported by research grant RG 991-A-3 from the National Multiple Sclerosis Society.

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