Measles Virus Polypeptides in Infected Cells Studied by Immune Precipitation and One-Dimensional Peptide Mapping

MICHAEL C. GRAVES

The Reed Neurological Research Center, University of California, Los Angeles, California 90024

Received 27 October 1980/Accepted 16 January 1981

Measles virus does not turn off host cell polypeptide synthesis, making it difficult to precisely identify the polypeptides specified by the virus during the infectious cycle. By using the technique of immune precipitation with measles-specific antisera, the host cell background has been eliminated, and new observations have been made concerning measles virus polypeptides H, P, NP, F, and M. The H polypeptide is first synthesized as a monomer which is processed by further glycosylation and by the formation of disulfide-bonded dimers. Polypeptide P (70,000 daltons) has been found to occur also as a 65,000-dalton molecule, P₂, and both forms of the molecule are equally phosphorylated. Polypeptide NP is processed from a cleavage-sensitive form (which undergoes cleavage during the process of isolation to form polypeptide 6 [41,000 daltons]) to a form which is resistant to this cleavage. The fusion and hemolysin polypeptide is first found in the cells as a 55,000-dalton precursor, F₀, which is clearly resolved from the NP polypeptide on gel electrophoresis. The measles virus F₀ protein identified in previous reports had not been resolved from the 60,000-dalton NP polypeptide. The M protein occurs in the infected cells as two distinct bands, and, as in the case of Sendai virus, one of these two M protein bands represents a phosphorylated form of the other.

Measles virus infection does not turn off cell protein synthesis, making it difficult to precisely identify the polypeptides specified by the virus during the infectious cycle and how they may be processed from one form to another. In this report, the technique of immune precipitation was used in the study of measles virus polypeptide synthesis. In this way, the background of host cell polypeptides was eliminated, and significant improvement in the detection and resolution of the viral polypeptides was achieved. Previous studies of the virus and of virus-infected cells have indicated the following measles polypeptides: L (200,000 daltons) and P (70,000 daltons), which are analogous to polymerase proteins of other paramyxoviruses; NP (60,000 daltons), the nucleocapsid subunit protein; H (80,000 daltons), the hemagglutinin and larger of the two viral glycoproteins; F, the fusion and hemolysin glycoprotein which is present on the virion as F₁, a 40,000-dalton polypeptide, and F₁, a 16,000- to 18,000-dalton glycopolypeptide, joined by disulfide linkages; and M, a 38,000-dalton nonglycosylated membrane protein (6, 16, 19, 22-24). The technique of immune precipitation has been previously used to demonstrate the absence of antibody to measles virus M protein in patients with subacute sclerosing panencephalitis (7, 8; C. E. Machamer, E. C. Hays,

and H. J. Zweerink, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1979, S131, p. 261) and has also been useful and has also been useful in elucidating the relationship of measles virus to canine distemper virus (9). In this work the technique was used to study the synthesis and processing of the viral polypeptides in the infected cell.

MATERIALS AND METHODS

Cells. The Vero cell line, originally from Flow Laboratories, Rockville, Md., and the HeLa cell line, originally from Michael Oldstone, Scripps Clinic and Research Foundation, La Jolla, Calif., were both obtained from Purnell W. Choppin, Rockefeller University, New York, N.Y., and were grown in Dulbecco-modified Eagle medium (DMEM) (Flow Laboratories) with 10% fetal calf serum.

Virus. The Edmonston strain of measles virus was grown in Vero cells to a titer of 2×10^7 PFU/ml as previously described (6).

Infection of cells. Monolayers of HeLa cells in 3.5-cm plastic dishes were infected at a multiplicity of infection of 10 and incubated for 16 to 24 h at 37°C with 10 µg of SV4814 (carbobenzoxymethyl-D-phenylalanyl-D-phenylalanyl-nitro-L-arginine) per ml. As previously described, SV4814 protects the cells from virus-induced fusion, allowing prolonged survival of the infected cells so that pulse-chase experiments can be done, and has no effect on the synthesis of measles virus polypeptides (6).

Isotopic labeling of the cells. For pulse-chase

experiments, cells were washed and incubated with leucine-free DMEM for 30 min before being labeled with 50 μ Ci of [3 H]leucine in 0.5 ml of leucine-free DMEM per dish for 30 min, and the chase was accomplished by removing the radioactive medium and incubating cells with complete medium for 3 h, as described (6).

In phosphorylation studies (Fig. 3), the labeling was carried out just as described above, except that 100 μ Ci of [32 P]phosphate in 1 ml of phosphate-free DMEM was used, and companion cultures were labeled with 100 μ Ci of [3 H]leucine in 1 ml of leucine-free DMEM also for 2 h. For double-isotope experiments, cells (at 6 h postinfection) were labeled with 400 μ Ci of [32 P]phosphate in 0.9 ml of phosphate-free DMEM and 0.1 ml of complete DMEM for 14 h, after which time the 32 P-containing medium was removed, and the cells were labeled with [35 S]methionine for 2 h as described above.

Immune precipitation. The *Staphylococcus aureus* protein A method (11), as modified by Hall and Choppin (7), was used. Briefly, cell extracts in RIPA buffer (5), 1 ml per dish, were clarified by centrifugation and then incubated with antiserum for 2 to 3 h on ice. The resulting immune complexes were absorbed on *S. aureus* protein A linked to Sepharose 4CLB beads, which were washed five times in RIPA buffer and then analyzed by electrophoresis (6).

Sera from nine individuals, either vaccinees or patients with measles or atypical measles, were screened to select the antiserum which gave the maximum yield of all the recognized viral polypeptides. There was a wide variation in the yield of each particular viral polypeptide obtained with the various sera. For this report, a serum was selected which gave the maximum yield of each of the recognized viral polypeptides. As controls, nonimmune prevaccination sera were available in two cases. Under the conditions of these experiments, there was essentially no detectable precipitation of viral polypeptides by the preimmune sera, nor was there any detectable precipitation of polypeptides from uninfected cells reacted with measles-immune sera. In the case of 32 P labeling experiments, however, there was a high host cell background, which was minimized by preabsorbing the cell extracts with Sepharose 4CLB beads and then with nonimmune immunoglobulin on *S. aureus* protein A-Sepharose 4CLB beads, each for 20 min, and by washing the final immune precipitate with an extract of nonradioactive HeLa cells in RIPA buffer. Using these added steps, a reasonably low background for 32 P labeling experiments was obtained (see Fig. 3a).

To determine the optimum ratio of antiserum to infected-cell lysate, titration experiments were carried out. The results indicated that a ratio of from 0.1 to 1.0 μ l of serum to 250 μ l of cell lysate represented antibody excess and was optimum for maximum visualization of each of the viral polypeptide bands. This ratio was used in this study. The use of larger ratios of antibody to infected-cell lysate gave decreased yields of polypeptides H and F₀, a prozone effect which was not seen for the other viral proteins.

Microsomal preparation. For the experiment in Fig. 1, the pulse-labeled cells were disrupted by Dounce homogenization, and a crude microsomal

preparation was prepared as described by Robbins et al. (18) by sedimenting nuclei at 700 $\times g$ for 7 min and removing soluble proteins by pelleting at 100,000 $\times g$ for 1 h. The washed pellet was used for electrophoretic analysis. To rule out loss or specific transport of viral polypeptides into the soluble or nuclear fractions, these too were monitored by electrophoresis.

Polyacrylamide gel electrophoresis. As described previously (6), gels were 10% acrylamide for polypeptide resolution and 17% for peptide mapping (14), both with a ratio of acrylamide to bisacrylamide of 37.5:1.

Autoradiography and fluorography. Gels were exposed without (Fig. 3b) or with PPO (2,5-diphenyloxazole) before drying, as reported (1, 14). For quantitation of double-isotope experiments, polypeptide bands were excised and solubilized in 0.3 ml of Protosol containing 5% water, and liquid scintillation counting was used to estimate 32 P and 35 S. Spillover was less than 0.5% for 35 S into the 32 P channel and 7.5% for 32 P into the 35 S channel. After correction for isotope decay and subtraction of background values, ratios of 32 P counts to 35 S counts were calculated.

Sera. Serum samples were generously supplied by James Cherry, Department of Pediatrics, University of California, Los Angeles, Calif.

Chemicals and isotopes. SV4814 was the generous gift of E. D. Nicolaides, Parke, Davis & Co., Ann Arbor, Mich. [3 H]leucine, [35 S]methionine, and sodium [32 P]phosphate were from Amersham Corp., Arlington Heights, Ill. Chymotrypsin was from Worthington Diagnostics, Freehold, N.J. *S. aureus* V8 protease and chemicals for polyacrylamide gels were from Miles Laboratories, Inc., Elkhart, Ind. *S. aureus* protein A-linked Sepharose 4CLB was from Pharmacia Fine Chemicals, Piscataway, N.J. Tosylsulfonylphenylalanyl chloromethyl ketone, tosylsulfonyllysyl chloromethyl ketone, and phenylmethylsulfonyl fluoride were from Sigma Chemical Co., St. Louis, Mo.

RESULTS AND DISCUSSION

Identification of the uncleaved fusion protein precursor, F₀. To study the processing of the viral fusion glycoprotein, a pulse-chase experiment was carried out, and the labeled polypeptides were analyzed in a crude microsomal preparation. This technique was shown to significantly reduce the background of host cell polypeptides without significant loss of viral proteins. Polypeptides of microsomal preparations from uninfected cells (U), infected pulse-labeled cells (I₀), and pulse-chased cells (I₃) were analyzed on a 10% gel (Fig. 1a). The viral polypeptides of Fig. 1a were excised by using the superimposed X-ray film as a guide, rehydrated, and then analyzed by peptide mapping on a 17% polyacrylamide gel (2, 14). In addition to the previously recognized viral polypeptides L, H, P, NP, F₁, and M (6), Fig. 1a shows a band labeled F₀ which migrated as a 55,000-dalton band just below NP. This band is identified as F₀ because (i) it appeared in the pulse and disappeared in

FIG. 1. (a) Measles virus-infected HeLa cells were pulse-labeled with [3 H]leucine for 30 min and then chased with DMEM for 3 h. A microsome preparation from the infected cells was prepared, dissolved in sample buffer, and electrophoresed in a 10% polyacrylamide gel. A fluorogram of this gel is shown. U, Uninfected preparation; I₀, infected, pulse-labeled preparation; I₃, pulse-labeled preparation chased with nonradioactive medium for 3 h. (b) One-dimensional peptide mapping in a 17% polyacrylamide gel of the various viral polypeptide bands shown in (a). The bands were cut out of the dried gel by using the superimposed fluorogram as a guide and were digested in the gel with chymotrypsin, 7.5 μ g per slot (2, 13). Bands from the corresponding region of the uninfected lane were included as controls (U), and in the case of F₀ and F₁, bands from corresponding regions of uninfected (U), infected, pulse-labeled (I₀), and infected, pulsed and chased (I₃) lanes were included. The digest products common between F₀ and F₁ are noted with horizontal lines.

the chase, just as F₁ was not present in the pulse but appeared in the chase; (ii) peptide mapping shows partial similarity between F₀ and F₁ (Fig. 1b); and (iii) it comigrated with the unreduced, cleaved form of the fusion protein, F_{1,2} (Fig. 2a, discussed below). The previously identified 60,000-dalton F₀ (6) may represent a different state of glycosylation of the 55,000-dalton F₀ identified here. Differences in the electrophoresis system may explain why others reported F_{1,2} to comigrate with the 60,000-dalton NP polypeptide (10, 16).

To more completely eliminate the background of host cell polypeptides, viral polypeptides from a pulse-chase experiment were analyzed after immune precipitation with measles antiserum (Fig. 2a). The electrophoresis was done both with and without reducing agent so as to demonstrate the 55,000-dalton molecule F_{1,2}, which has been shown to consist of F₁ and F₂ joined by disulfide bonds (6, 10, 16). The reduced lanes of Fig. 2a show that the previously recognized viral polypeptides as well as the 55,000-dalton F₀ were all recognized by measles immune serum. The pattern of viral polypeptides seen on the unreduced section of Fig. 2a is slightly different from that seen on reduced gels (Fig. 1a), and unre-

duced polypeptides were therefore excised from the dried gel, rehydrated, and reelectrophoresed under reducing conditions for identification (Fig. 2b). Note that F₀ from the pulsed, unreduced lane of Fig. 2a yielded only F₀ upon reduction (lane 2, Fig. 2b), but the corresponding band from the chased, unreduced lane, F_{1,2}, yielded F₁ and F₂ as well (Fig. 2b, lane 3), indicating that the 55,000-dalton F₀ polypeptide identified in Fig. 1 was being processed into F_{1,2} during the chase. Further evidence of the relationship of F₀ as a precursor to F_{1,2} was obtained by peptide mapping. As shown in Fig. 1b, the digest patterns of F₀ and F₁ were only partially similar, perhaps because F₀ contains carbohydrate and F₁ does not. However, F_{1,2} from chased, unreduced preparations would be expected to be similar to F₀ in carbohydrate content in the absence of reducing agent, since the two cleavage fragments, F₁ and F₂, remain connected by disulfide bonds (10, 16). Peptide maps were therefore carried out in the absence of reducing agents, and under these conditions the digest patterns of F₀ and F_{1,2} were nearly identical (Fig. 2c). This experiment does not by itself eliminate the possibility that a comigrating 55,000-dalton polypeptide completely unrelated to F₀ and F_{1,2} exists and is the

FIG. 2. (a) Fluorogram of a 10% polyacrylamide gel showing pulse-chase-labeled viral polypeptides immune precipitated from infected cells, run with or without reducing agent. (b) Reelectrophoresis after reduction of the unreduced polypeptides. The following bands were cut out from the unreduced lanes of (a), rehydrated, soaked in sample buffer containing 1% 2-mercaptoethanol, placed in the slots of a second 10% gel, and reelectrophoresed: 1, $H_{unr.}$; 2, F_0 (from pulse lane); 3, $F_{1,2}$ (from chase lane); 4, M; 5, M_2 (not visualized in Fig. 6a); 6, M (from reduced pulse lane of Fig. 6a); and 7, P. This gel in lane 7 was used to identify the bands in the unreduced gel. (c) One-dimensional peptide maps of viral polypeptides 6 and NP, digested with 0.1 μ g of *S. aureus* protease V8, and, from a separate experiment, F_0 and $F_{1,2}$, which were digested in the absence of reducing agent.

basis for the similarities of peptide maps between the 55,000-dalton F_0 and $F_{1,2}$ bands and for the lack of complete similarity between maps of F_0 and F_1 . A precedent for this has been found in canine distemper virus-infected cells, where we have found a nucleocapsid polypeptide cleavage product, NP_2 , which migrates at precisely this 55,000-dalton region and which could be easily confused with F_0 . The existence of such a non-F, 55,000-dalton polypeptide in measles-infected cells is unlikely for two reasons. First, peptide maps do not show similarity between F_0 and NP or any larger viral protein (Fig. 1), whereas the canine distemper virus 55,000-dalton polypeptide shows a digest pattern identical to that of NP (unpublished data). Second, it seems unlikely that a postulated non-F 55,000-dalton polypeptide would imitate the behavior of the F_0 in pulse-chase experiments. Taking the available results of three different kinds of experiments which have been done (pulse-chase, the comigrating of F_0 and $F_{1,2}$, and the peptide maps), the most reasonable conclusion is that a 55,000-dalton precursor, F_0 , is initially synthesized and gives rise to F_1 and F_2 by proteolytic processing.

Identification of polypeptide 6 as a cleavage product of newly synthesized nucleocapsid polypeptide NP. Polypeptide 6 is a 41,000-dalton band which nearly comigrates with F_1 and therefore could be easily confused with F_1 . The two proteins are clearly distinguished by the characteristic behavior of F_1 in pulse-chase experiments and on unreduced gels and by peptide mapping, which shows similar digest patterns for NP and 6. Since 6 is not seen in whole-cell preparations lysed in electrophoresis sample buffer (Fig. 1a and similar unpublished results), but is seen in preparations lysed in RIPA buffer (data not shown), it would appear that 6 is an artifact of the extraction procedure resulting from an endogenous specific cleavage of NP which occurs in the presence of the mild detergents of RIPA buffer. Polypeptide 6 is formed only in pulse-labeled preparations and not in chased preparations (Fig. 2a, unreduced lanes), indicating that the nucleocapsid protein must undergo a processing event during the chase which changes it from a cleavage-sensitive to a cleavage-resistant form. Experimental manipulations such as the use of different solubilization detergents and of different pro-

tease inhibitors such as tosylsulfonyllysyl chloromethyl ketone and tosylsulfonylphenylalanyl chloromethyl ketone have not eliminated the generation of polypeptide 6. Another NP polypeptide cleavage product of approximately 45,000 daltons has also been identified by peptide mapping (data not shown) and is referred to as polypeptide 5 (Fig. 2a and 3a). The propensity of polypeptide NP to undergo proteolytic cleavages has been previously discussed (14, 17, 20).

Processing of hemagglutinin polypeptide H. It has been previously shown that the 80,000-dalton H polypeptide occurs on the virion as a higher-molecular-weight disulfide-bonded dimer (10). It can be seen in Fig. 2a (unreduced lanes) that H is first synthesized in the cells as a monomer (labeled H_0) which is processed during the chase to form the disulfide-bonded dimer (or possibly trimer) H_{unr} . The identity of H_{unr} was confirmed by reelectrophoresis under reducing conditions (Fig. 2b, lane 1). In pulse-chase experiments, the apparent molecular weight of

polypeptide H increased during the chase (Fig. 1a and 2a), and with longer labeling times (2 h) both forms of H could be visualized (Fig. 3b). Since pulse-chase experiments showed conversion of H_0 to H (Fig. 1a and 2a) and since the peptide maps were similar (Fig. 4), H is probably a more heavily glycosylated form of H_0 . H_0 was not found as a dimer in unreduced preparations, and completion of glycosylation of H may be required for dimerization by disulfide bonding, but it is also possible that glycosylation and dimerization are simply parallel processing events.

Identification of the 65,000-dalton polypeptide P_2 with the 70,000-dalton phosphoprotein. It has been previously reported that a 65,000-dalton polypeptide is found in measles virus-infected cells which have been treated with protease inhibitors or in cells in which protein synthesis has been synchronized with hypertonic salt (23). Peptide mapping was not reported, but the 65,000-dalton polypeptide was thought to

FIG. 3. (a) Measles virus-infected cells labeled for 2 h with [^3H]leucine (^3H) or with 100 μCi of sodium [^{32}P]phosphate per ml (^{32}P). This experiment indicates phosphate labeling of P, NP, and M_1 proteins. Band 5 is an NP cleavage product. (b) Infected culture was simultaneously labeled with [^{35}S]methionine and sodium [^{32}P]phosphate as described in the text. Excised bands were analyzed by liquid scintillation counting for ^{32}P and ^{35}S . Ratios of ^{32}P to ^{35}S counts for the bands are as follows: H, 0.043; P, 0.482; P_2 , 0.423; NP, 0.227; M_1 , 0.350; M_2 , 0.093. Values are means of two experiments.

FIG. 4. Peptide map of some of the measles virus polypeptides, digested with 1.0 μg of *S. aureus* protease V8 and resolved on a 17% polyacrylamide gel. H_0 is the nondimerized precursor to polypeptide H. ^{32}P , Maps of polypeptides P and P_2 labeled with ^{32}P . TPCK, Map of the 65,000-dalton polypeptide found in cells treated with tosylsulfonylphenylalanyl chloromethyl ketone.

represent F_0 because of the conditions under which it appeared (23). A similar 65,000-dalton band was seen in Fig. 1a, 2a, and 3 of this paper, and peptide maps identified it with the 70,000-dalton viral phosphoprotein P. To further compare the present results with those reported by Vainionpää (23), this author's experiments were reproduced. It was found that either by use of a protease inhibitor (0.1 mM tosylsulfonylphenylalanyl chloromethyl ketone) or by treating cells for 45 min with hypertonic salt (300 to 400 mM NaCl), the synthesis of the 65,000-dalton polypeptide could be markedly enhanced while that of the 70,000-dalton polypeptide P was decreased (data not shown), just as reported (23). However, the 65,000-dalton polypeptide formed under these conditions was identical in its gel migration (data not shown) and in its peptide map (shown in Fig. 4) with the P and P_2 polypeptides reported in this paper. Since P is a phosphoprotein, it was postulated that P_2 might represent a non-phosphorylated precursor to P. A double-isotope experiment was carried out by labeling the same infected culture with [^{32}P]-phosphate and with [^{35}S]methionine. Polypeptide bands were located using the superimposed X-ray film, excised, solubilized in Protosol, and analyzed by liquid scintillation counting, and the data were expressed as a ratio of ^{32}P counts to ^{35}S counts. Similar levels of phosphorylation were found for both P and P_2 (ratios of 0.482 and 0.423, respectively). Also, partial digest peptide maps of ^{32}P -labeled P and P_2 were similar (Fig. 4), indicating that the digestion fragments to which the phosphate was bound were no different for P and P_2 . These results indicate that a difference in phosphorylation is not likely to explain the migration difference of the two forms of the phosphoprotein and that P_2 is not the unphosphorylated precursor to P. Thus, P and P_2 differ only in their apparent molecular weight as determined by electrophoresis in polyacrylamide gels. The possibility that P_2 may arise by proteolytic cleavage of a small (5,000-dalton) fragment from P is made unlikely by the lack of observation of conversion of P to P_2 in pulse-chase experiments and by the peptide maps, which show no migration differences in the partial digest fragments (Fig. 1b and 4). The observation that protease inhibitors actually lead to increased amounts of the 65,000-dalton P_2 is also inconsistent with this idea (23). It is suggested that P_2 differs from P in its secondary structure, since in unreduced gels P and P_2 comigrate as a 65,000-dalton band which can be shown to consist of both proteins by reduction and reelectrophoresis (Fig. 2a and b, lane 7). One possibility is that both P and P_2 are in a compact conformation which migrates as a 65,000-dalton mole-

cule. Reducing agents break intrachain disulfide bonds and lead to the unfolding of P to a conformation which now migrates as a 70,000-dalton molecule on gels. P_2 , however, is resistant to this unfolding. An analogous situation can be found in the case of the NS polypeptide of vesicular stomatitis virus, where conformational changes have been suggested to explain gel migration differences in the NS phosphoprotein of the class E temperature-sensitive mutants (3, 15).

The present data do not entirely exclude the possibility that P_2 is a cleavage product of P or that it occurs as an artifact of the extraction procedure; however, since it is possible to experimentally change the ratio of P to P_2 by use of hypertonic salt or tosylsulfonylphenylalanyl chloromethyl ketone, these two bands may represent alternate forms of the protein actually present in the cell which differ in biological activities. Future experiments will be aimed at using this observation to delineate the function of the P polypeptide. In the case of vesicular stomatitis virus, alternate forms of an analogous phosphoprotein, NS, have been reported (12, 15), and the suggestion has been made that phosphorylation of viral core proteins may be a control mechanism which determines whether the virion polymerase makes genomic-length RNA for replication or mRNA (21).

Identification of two forms of the M polypeptide. In Sendai virus-infected cells, an alternate form of the M polypeptide has been reported (13). This polypeptide, B, has been shown to represent a phosphorylated form of the Sendai virus M protein which is formed transiently in the infected cell, and the authors comment upon similar observations for the measles virus M protein (9). Because of closely migrating host cell polypeptides, it is difficult to resolve with certainty the two forms of measles virus M protein, but the use of immune precipitation has eliminated this problem. Although not seen in all experiments, it is possible to demonstrate two distinct bands, M_1 and M_2 , which have identical peptide maps (Fig. 3b and 4). It is also possible to demonstrate phosphorylation of the M_1 protein band (Fig. 3), but since only about 2% of the total viral polypeptide ^{32}P label is in M (data obtained by densitometry of Fig. 3a and other similar experiments), phosphorus labeling of the M protein was seen only in the most favorably labeled ^{32}P preparations. The double-isotope experiment depicted in Fig. 3b, in which the same infected culture was labeled with sodium [^{32}P]-phosphate and [^{35}S]methionine, showed significant phosphate labeling of the upper M protein band only. As noted above, it has been difficult to obtain adequate ^{32}P labeling of the M polypeptide, and further characterization has not

been achieved as it has for the ^{32}P -labeled P protein. Others have not found phosphorylation of the M polypeptide (19, 24), perhaps because of inadequate total ^{32}P labeling of the viral proteins, but it has been reported by at least two groups (4, 9). Besides phosphorylation, a proteolytic cleavage could be suggested as an explanation for the two bands. Vainionpää reported finding that in cells treated with tosylsulfonyl-lysyl chloromethyl ketone the molecular mass of M is 1,000 to 2,000 daltons higher than in untreated cells and suggested that M may be trypsin sensitive (23). Although such a cleavage has not been ruled out, the ^{32}P labeling data indicate that the measles virus M protein undergoes phosphorylation in the infected cell and is in this respect analogous to that of Sendai virus.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI-16029 from the National Institutes of Health.

I thank Evelyn Price for excellent technical assistance and Jack G. Stevens and Debi P. Nayak for helpful discussions.

LITERATURE CITED

- Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**:83-88.
- Cleveland, D. W., S. G. Fishcher, N. W. Kirschner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecylsulfate and analysis by gel electrophoresis. *J. Biol. Chem.* **252**:1102-1106.
- Evans, D., C. R. Pringle, and J. F. Szilagyi. 1979. Temperature-sensitive mutants of complementation group E of vesicular stomatitis virus New Jersey serotype possess altered NS polypeptides. *J. Virol.* **31**:325-333.
- Fujinami, R. S., and M. B. A. Oldstone. 1980. Alterations in expression of measles virus polypeptides by antibody: molecular events in antibody induced antigenic modulation. *J. Immunol.* **125**:78-85.
- Gilead, Z., Y. Jeng, W. S. M. Wold, K. Sugawara, H. M. Rho, M. I. Harter, and M. Green. 1976. Immunological identification of two adenovirus 2 induced early proteins possibly involved in cell transformation. *Nature (London)* **264**:163-166.
- Graves, M. C., S. M. Silver, and P. W. Choppin. 1978. Measles virus polypeptide synthesis in infected cells. *Virology* **86**:154-163.
- Hall, W. W., and P. W. Choppin. 1979. Evidence for lack of synthesis of the M polypeptide of measles virus in brain cells in subacute sclerosing panencephalitis. *Virology* **99**:443-447.
- Hall, W. W., R. A. Lamb, and P. W. Choppin. 1979. Measles and SSPE virus proteins: lack of antibodies to the M protein in patients with subacute sclerosing panencephalitis. *Proc. Natl. Acad. Sci. U.S.A.* **76**:2047-2051.
- Hall, W. W., R. A. Lamb, and P. W. Choppin. 1980. The polypeptides of canine distemper virus: synthesis in infected cells and relatedness to the polypeptides of other morbilliviruses. *Virology* **100**:433-499.
- Hardwick, J. M., and R. H. Bussell. 1978. Glycoproteins of measles virus under reducing and nonreducing conditions. *J. Virol.* **25**:687-692.
- Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A antibody absorbent: parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* **115**:1617-1624.
- Kingsford, L., and S. U. Emerson. 1980. Transcriptional activities of different phosphorylated species of NS protein purified from vesicular stomatitis virions and cytoplasm of infected cells. *J. Virol.* **33**:1097-1105.
- Lamb, R. A., and P. W. Choppin. 1977. The synthesis of Sendai virus polypeptides in infected cells. III. Phosphorylation of polypeptides. *Virology* **81**:382-397.
- Lamb, R. A., and P. W. Choppin. 1978. Determination by peptide mapping of the unique polypeptides in Sendai virions and infected cells. *Virology* **84**:469-478.
- Lesnaw, J. A., L. R. Dickson, and R. H. Curry. 1979. Proposed replicative role of the NS polypeptide of vesicular stomatitis virus: structural analysis of an electrophoretic variant. *J. Virol.* **31**:8-16.
- Mountcastle, W. E., and P. W. Choppin. 1976. A comparison of the polypeptides of four measles virus strains. *Virology* **78**:463-474.
- Mountcastle, W. E., R. W. Compans, H. Lackland, and P. W. Choppin. 1974. Proteolytic cleavage of the subunits of the nucleocapsid of the paramyxovirus, simian virus 5. *J. Virol.* **14**:1253-1261.
- Robbins, P. W., S. S. Krag, and T. Liu. 1977. Effects of UDP-glucose addition on the synthesis of mannosyl lipid linked oligosaccharides by cell free fibroblast preparations. *J. Biol. Chem.* **252**:1785.
- Robbins, S. J., and R. H. Bussell. 1979. Structural phosphoproteins associated with purified measles virions and cytoplasmic nucleocapsids. *Intervirology* **12**:96-102.
- Sprague, J., L. J. Eron, A. S. Seifried, and J. B. Miltien. 1979. Cell-free synthesis of measles virus proteins. *J. Virol.* **32**:688-691.
- Testa, D., P. K. Chanda, and A. K. Banerjee. 1980. In vitro synthesis of the full-length complement of the negative strand genome RNA of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. U.S.A.* **77**:294-298.
- Tyrrell, D. L. J., and E. Norrby. 1978. Structural polypeptides of measles virus. *J. Gen. Virol.* **39**:219-229.
- Vainionpää, R. 1979. Measles virus specified polypeptides in infected cells. *Arch. Virol.* **60**:239-248.
- Wechsler, S. L., and B. N. Fields. 1978. Intracellular synthesis of measles virus-specified polypeptides. *J. Virol.* **25**:235-297.