Polypeptide Synthesis in Simian Virus 5-Infected Cells

RICHARD W. PELUSO,* ROBERT A. LAMB, AND PURNELL W. CHOPPIN

The Rockefeller University, New York, New York 10021

Polypeptide synthesis in three different cell types infected with simian virus 5 has been examined using high-resolution polyacrylamide slab gel electrophoresis, and all of the known viral polypeptides have been identified above the host cell background. The polypeptides were synthesized in infected cells in unequal proportions, which are approximately the same as they are found in virions, suggesting that their relative rates of synthesis are controlled. The nucleocapsid polypeptide (NP) was the first to be detected in infected cells, and by 12 to 14 h the other virion structural polypeptides were identified, except for the polypeptides comprising the smaller glycoprotein (F). However, a glycosylated precursor (F_0) with a molecular weight of 66,000 was found in each cell type, and pulse-chase experiments suggested that this precursor was cleaved to yield polypeptides F_1 and F_2. No other proteolytic processing was found. In addition to the structural polypeptides, the synthesis of five other polypeptides, designated I through V, has been observed in simian virus 5-infected cells. One of these (V), with a molecular weight of 24,000, was found in all cells examined and may be a nonstructural viral polypeptide. In contrast, there are polypeptides present in uninfected cells that correspond in size to polypeptides I through IV, and similar polypeptides have also been detected in increased amounts in cells infected with Sendai virus. These findings, and the fact that the synthesis of all four of these polypeptides is not increased in every cell type, suggest that they represent host polypeptides whose synthesis may be enhanced upon infection. When a high salt concentration was used to decrease host cell protein synthesis in infected cells, polypeptides IV and (to a lesser extent) I were synthesized in relatively greater amounts than other cellular polypeptides, as were the viral polypeptides. The possibility that these polypeptides may play some role in virus replication is discussed.

The paramyxovirus simian virus 5 (SV5) has been shown to contain five major structural proteins, designated HN, NP, F, 5, and M (5, 23, 25). Two of the viral proteins, HN and F, are glycosylated (15), and the larger of these (HN) has been shown to possess both hemagglutinating and neuraminidase activities, whereas the smaller (F) is thought to be involved in hemolysis and cell fusion (34–36, 38). The F glycoprotein of Sendai virus and Newcastle disease virus (NDV), two other paramyxoviruses, has been shown to be derived by specific proteolytic cleavage of a precursor glycoprotein, designated F_0, which is accompanied by activation of the cell-fusing and hemolyzing activities of the virion and its ability to initiate infection (12, 14, 27, 32, 35, 37). Although biologically inactive virions containing the uncleaved F_0 glycoprotein have been found with Sendai virus and NDV, such virions have never been found with SV5. However, evidence for the existence of such a precursor has been obtained in that the SV5 F protein, as well as those of Sendai virus and NDV, has been found to consist of two subunits, F_1 and F_2, linked by disulfide bonds (33, 38).

The most abundant virion protein, NP, complexes with viral RNA to form the nucleocapsid (24), and the smallest protein, M, is thought to be associated with the inner surface of the viral envelope (23). The remaining major structural protein, 5, is thought to be involved in the virion RNA polymerase activity (E. Buetti and P. W. Choppin, submitted for publication), along with NP and possibly a viral protein present in small amount, L. The host cell protein actin (A) has recently been found in some paramyxovirions (39), but the significance of this is not yet clear.

Although much is known about the structure, composition, and functions of the components of SV5 and its interactions with cells in cytocidal and noncytocidal infections (6, 9, 11, 16–18), relatively little is known about the de-

tails of the synthesis of its proteins. This is due largely to the difficulties inherent in studying viral protein synthesis in a system in which host cell synthesis is not rapidly turned off (10, 13), a situation common with paramyxoviruses. Recently, it has been possible to study the synthesis of Sendai and influenza virus-induced proteins in infected cells by the use of [^{35}S]methionine labeling, high-resolution slab gel electrophoresis, and autoradiography (20, 21). This report describes SV5-induced protein synthesis in three different cell types. The time course of viral protein synthesis has been studied by pulse-labeling, and the processing of one of the viral glycoproteins (F) has been demonstrated in pulse-chase experiments with [^{3}H]-glucosamine.

MATERIALS AND METHODS

Cells. Monolayer cultures of a variant of the MDBK line of bovine kidney cells and the TC7 clone of CV-1 cells obtained from James Robb were grown in reinforced Eagle medium (REM) (1) with 10% fetal calf serum, as described previously (7). Primary cultures of chicken embryo fibroblasts (CEF) were grown in lactalbumin hydrolysate medium with 2% calf serum (21). Baby hamster kidney (BHK-21-F) cells for plaque assays were grown in REM with 10% calf serum and 10% tryptose phosphate broth as described previously (13). All cells were grown on plastic petri dishes.

Virus. Stock virus was grown in MDBK cells infected with the W3 strain of SV5 (6) at a multiplicity of ~1 PFU/cell. After a 2-h adsorption period at 37°C, the monolayers were washed with phosphate-buffered saline (PBS), and REM without serum was added. After 3 to 4 days at 37°C, the medium was collected and clarified at 3,000 rpm for 30 min in a Sorvall GSA rotor. Bovine serum albumin was added to a concentration of 1%, and the virus was frozen at −70°C. Plaque assays were performed as described previously (8).

Chemicals and isotopes. ^{3}H-labeled reconstituted protein hydrolysate was purchased from Schwarz Bio-Research, Orangeburg, N.Y.; [^{3}H]leucine and [^{3}H]glucosamine, from New England Nuclear Corp., Boston, Mass.; [^{35}S]methionine, from Amersham/Searle, Arlington Heights, Ill.; sodium dodecyl sulfate, from Gallard-Schlesinger, Carle Place, N.Y.; dithiothreitol and ammonia-free glycine, from Calbiochem, La Jolla, Calif.; and acrylamide and bisacrylamide, from Ames Co., Elkhart, Ind.

Growth and purification of isotopically labeled virus. MDBK cells were infected as described above; after adsorption, the monolayers were washed with PBS, and REM containing 5 μCi of [^{3}H]leucine per ml was added. After 3 to 4 days, the medium was harvested and clarified, and the virus was pelleted by centrifugation at 10,000 rpm for 2 h in a GSA rotor. The pellet was suspended in 10% potassium tartrate, homogenized in a Dounce homogenizer, and layered on a linear 15 to 35% (wt/wt) potassium tartrate gradient. After centrifugation at 22,000 rpm

in a Spinco SW27 rotor for 2 h, the virus band was collected and dialyzed against 0.0625 M Tris, pH 6.8. Purified virus was kept at −20°C.

Infection and labeling of cells. Confluent monolayers on 60-mm dishes were washed three times with PBS and inoculated with ~50 PFU/cell; after a 1-h adsorption, the monolayers were again washed three times, and 2 ml of Eagle medium was added. At various times after infection, the medium was removed and replaced with 1 ml of one of the following: (i) REM deficient in methionine and containing [^{35}S]methionine (10 μCi/ml); (ii) REM deficient in amino acids and containing a ^{3}H-labeled amino acid mixture (10 μCi/ml); (iii) REM deficient in glucose and containing [^{3}H]glucosamine (25 μCi/ml). After incubation at 37°C for periods as indicated in Results, the medium was removed, and the cells were washed twice with PBS and lysed in a solution consisting of 4% sodium dodecyl sulfate, 3% dithiothreitol, 40% glycerol, and 62.5 mM Tris, pH 6.8. Lysates were scraped from the dish and frozen at −20°C until processed for electrophoresis. For pulse-chase experiments, the radioactive medium was removed, the cells were washed three times with PBS, and REM was added to the dishes. At the appropriate times, the cells were harvested as described above.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was done using a slight modification of the procedure described previously (21); instead of an acrylamide-bisacrylamide ratio of 37.5:1, a ratio of 77:1 was used to obtain better resolution of infected cell lysates. Molecular weights of polypeptides were estimated by their migration relative to the following markers of known molecular weight: myosin, β-galactosidase, phosphorylase a, bovine serum albumin, catalase, ovalbumin, alcohol dehydrogenase, DNase I, carbonic anhydrase, α-chymotrypsinogen, and trypsin.

Autoradiography and fluorography. For detection of ^{3}H in gels, a fluorographic procedure (2) was employed using Kodak RP-Royal X-Omat film. For [^{35}S]methionine detection, dried gels were exposed to Dupont Cronex 2DC X-ray film. Fluorograms were processed by hand, and autoradiograms were processed using a Kodak 3.5-min X-Omat.

RESULTS

Structural proteins of SV5 virions. Figure 1 shows an autoradiograph of ^{3}H-labeled, purified virions grown in MDBK cells and subjected to slab gel electrophoresis. The five major polypeptides HN, NP, F$_1$, 5, and M have estimated molecular weights based on migration relative to markers in this gel system of ~70,000, 61,000, 52,000, 46,000, and 38,000, respectively. Two minor polypeptides, L and a protein that comigrates with cellular actin, with approximate molecular weights of 200,000 and 43,000, respectively, are also present. F$_2$, the smaller fragment derived by proteolytic cleavage of F$_0$, migrates with the buffer front in this gel and therefore is not resolved (38).

Polypeptide synthesis in SV5-infected MDBK

FIG. 1. *Autoradiograph of the polypeptides of SV5 virions separated by electrophoresis on a 10% polyacrylamide-sodium dodecyl sulfate (SDS) slab gel. Virions were labeled with [³H]leucine in MDBK cells, purified, disrupted with SDS and dithiothreitol, and subjected to electrophoresis as described in the text. Migration is from top to bottom.*

cells. In MDBK cells, infective virions begin to appear between 8 and 9 h postinfection (p.i.), and there is an exponential increase until 15 to 24 h p.i., followed by continuous virus pro-

duction for several days with only slight cytopathic effects. Virus yields at 36 to 72 h are usually in the range of 2×10^8 to 4×10^8 PFU/ml and 256 to 1,024 hemagglutination units/ml. Figure 2 shows an autoradiograph of a lysate of infected MDBK cells labeled at various times after infection with [³⁵S]methionine and subjected to electrophoresis. By 6 h p.i., the most prominent viral protein, NP, was distinguished against the host cell background, and by 12 to 14 h all the major virion polypeptides, with the exception of F_1 and F_2, were detected. In addition to these structural proteins, three polypeptides that are not found in virions appeared to be present in increased amounts in infected cells by 14 h. They are labeled I, II, and IV and have apparent molecular weights of ~99,000, 97,000, and 78,000, respectively. As discussed below, the available evidence suggests that these are cellular polypeptides whose synthesis is enhanced in infected cells. In addition to these polypeptides, infected MDBK cells contain a polypeptide with a molecular weight of ~24,000, designated V (see Fig. 5). This polypeptide ran off the gel shown in Fig. 2. The polypeptide migrating with a molecular weight of ~66,000, labeled F_0, will be shown below to be the precursor of polypeptides F_1 and F_2. The L protein was not detected in these cells, presumably because it is present in a very small amount and masked by comigration with host proteins. Although the amount of each viral protein has not been quantitated precisely, these and similar autoradiographs suggest that the virion polypeptides are synthesized in infected cells in unequal amounts and are present in cells in proportions similar to those found in virions.

Protein synthesis in primary CEF cells. In primary CEF cells, few SV5 virions are produced, i.e., $<10^5$ PFU/ml, there is no detectable hemagglutinin (<2 hemagglutination units), and no non-hemagglutinating particles are released. Therefore, the possibility of a defect in viral protein synthesis in CEF cells was investigated. Even though little or no virus is released from the cells, all the known viral proteins are synthesized (Fig. 3). The most abundant viral protein, NP, was detected before the other viral proteins, as early as 4 h p.i., and by 14 h all the known virion polypeptides except F_1 and F_2 were seen, including a small amount of the L protein which was just detectable at 14 to 18 h. In addition to the virion proteins, the glycoprotein precursor, F_0, was clearly seen in these autoradiographs. As shown above in MDBK cells, there were also large polypeptides whose synthesis was increased in these cells and which were not present in purified virions.

4U 4I 6U 6I 8U 8I 10U 10I 12U 12I   14U 14I 16U 16I 18U 18I
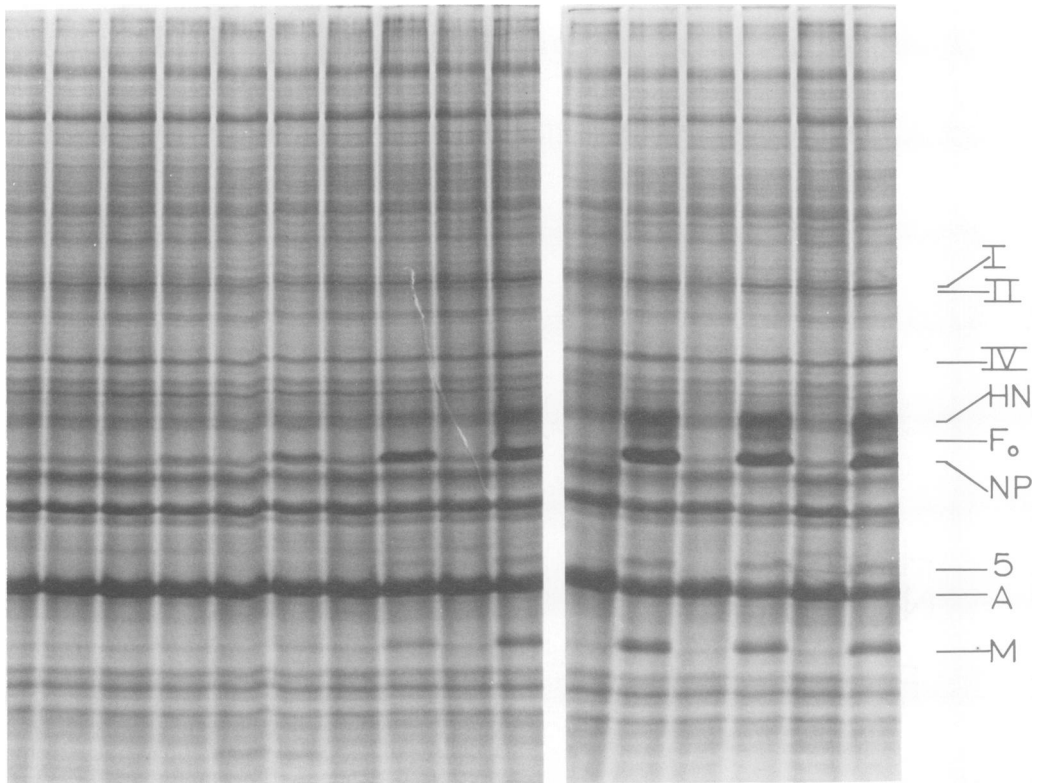

FIG. 2. *Time course of synthesis of SV5 polypeptides in MDBK cells. Cells were infected and labeled with [*^{35}*S]methionine for 60 min at various times after infection as indicated. In this and subsequent figures the numbers refer to the time after infection at which the label was added. I, Infected cell lysates; U, uninfected cell lysates. Whole-cell lysates were prepared for electrophoresis and autoradiography as described in the text. The polypeptides of unlabeled virions were included on each gel as markers.*

These are designated I, II, IV, and V, and their apparent molecular weights were similar in the two cell types. However, there was one polypeptide, III (molecular weight, ~86,000), that was detected in CEF but not in MDBK cells.

Protein synthesis in the CV-1 line of monkey kidney cells. Virus-specific protein synthesis was examined in the TC7 clone of CV-1 cells, which exhibit contact inhibition of growth (30), to determine if synthesis of minor viral proteins would be more easily detected in these cells. Figure 4 shows the results obtained with CV-1 cells pulse-labeled at various times after infection. In addition to the major virion structural polypeptides and F_0, polypeptides IV and V were clearly seen in these cells, and the L protein, though still present in a small amount, was detected at 12 to 18 h in these cells. No other precursors or minor proteins were seen.

Attempts to diminish host protein synthe-

sis. To obviate further the inherent difficulties in studying viral protein synthesis in cells in which there is no inhibition of host cell protein synthesis, attempts were made to selectively reduce the rate of synthesis of cellular proteins. Neither growing cells at 33°C nor the use of actinomycin D (0.05 to 5 μg/ml) was successful in this regard; however, the exposure of MDBK cells to hypertonic conditions prior to and during the labeling period, a procedure that causes a decrease in the initiation of cellular protein synthesis (28, 31), resulted in some degree of a selective inhibition of host synthesis. The viral polypeptides HN, F_0, NP, 5, and M, although synthesized at decreased rates as compared to cells not exposed to high salt, were prominent against the inhibited host cell background (Fig. 5, cf. Fig. 2). Figure 5 also shows that the synthesis of polypeptides I and IV appeared to be less inhibited in infected cells than in unin-

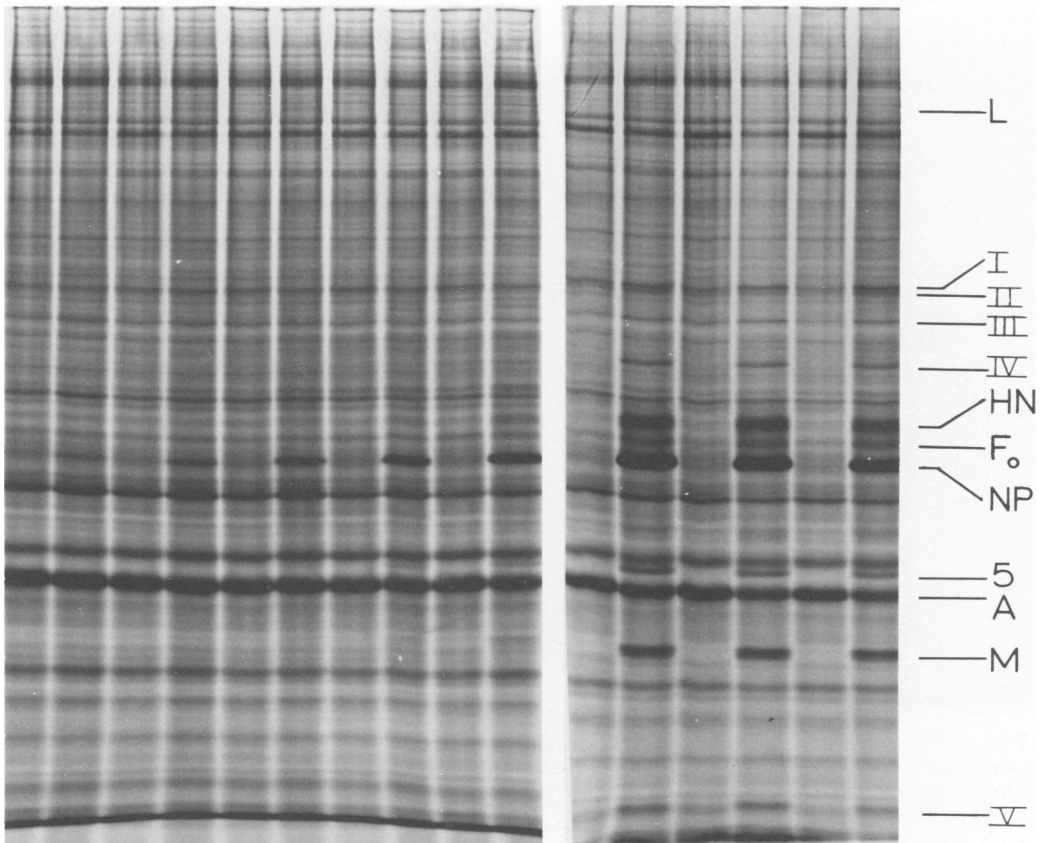4U 4I 6U 6I 8U 8I 10U 10I 12U 12I   14U 14I 16U 16I 18U 18I

FIG. 3. *Time course of SV5 polypeptide synthesis in primary CEF cells. Cells were labeled for 60 min with [³⁵S]methionine at the times indicated and processed for electrophoresis and autoradiography as described for Fig. 2.*

fected cells. This becomes apparent if one compares the synthesis of these polypeptides, particularly IV, with those of other cellular proteins whose rates of synthesis appeared similar in infected and uninfected cells. Thus, high salt appeared to cause less inhibition not only of viral polypeptides, but also of two presumed cellular polypeptides whose synthesis was enhanced in infected cells. This finding raises the possibility that these proteins may play a role in virus replication.

Figure 5 also emphasizes the value of methionine as a label in studies of SV5 proteins, since the labeling patterns of viral polypeptides obtained with the tritiated amino acid mixture in these experiments was similar to that obtained with methionine (cf. Fig. 2).

Comparison of polypeptide synthesis in SV5 and Sendai virus-infected cells. To investigate further the origin of polypeptides I through V, whose rates of synthesis in infected cells appeared to be greater than that in uninfected cells, the patterns obtained upon infection with SV5 and Sendai virus were compared. Polypeptides that correspond to polypeptides I through IV in SV5-infected cells were also found in Sendai virus-infected cells (Fig. 6). This finding, in addition to the fact that proteins with similar electrophoretic mobilities were found in uninfected cells, provides suggestive evidence that these are host polypeptides whose synthesis is enhanced after viral infection rather than virus-coded polypeptides. However, polypeptide V (molecular weight, ~24,000) of SV5-infected cells does not correspond to a protein in Sendai virus-infected cells, nor does polypeptide C (molecular weight, ~22,000) in Sendai virus-infected cells have a counterpart in
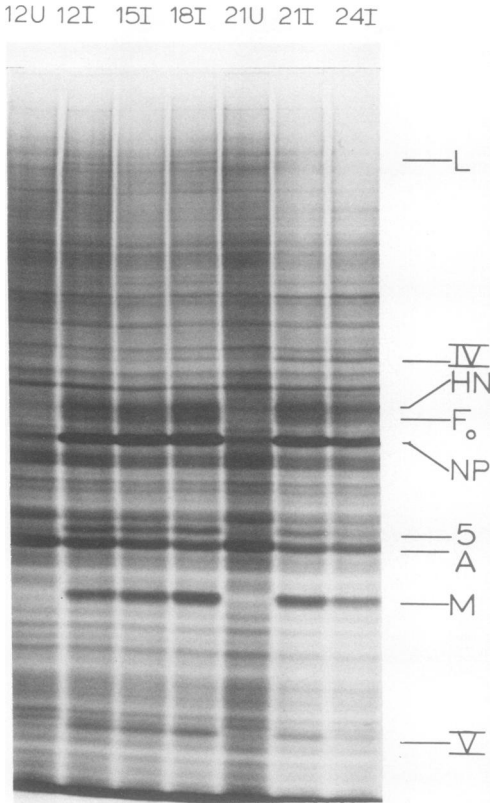
FIG. 4. SV5 polypeptide synthesis in CV-1 cells. At the times indicated, the cells were labeled with [³⁵S]methionine for 60 min and processed for electrophoresis and autoradiography.

SV5-infected cells. This suggests that polypeptide V may be a virus-specific, nonstructural polypeptide, as was previously suggested for polypeptide C in Sendai virus-infected cells (21).

Pulse-chase experiments in SV5-infected cells. In the above experiments in three different cell types, a glycoprotein corresponding to F_1 was not detected. However, another polypeptide that migrated slightly faster than HN was consistently found. By analogy to Sendai virus (21, 35, 37, 38), this was thought to be F_0. This precursor has never been found on SV5 virions; therefore, it is presumably processed by proteolytic cleavage before release of the virus in all cells thus examined, an assumption supported by the recent findings that the F protein of SV5, as well as those of Sendai virus and NDV, consists of two disulfide-linked polypeptides, F_1 and F_2 (38). To investigate whether the polypeptide observed in SV5-infected cells is such a precursor, pulse-chase experiments were performed in all three cell types. Figure 7

shows a representative experiment in which CEF cells were pulsed for 60 min at 17 h p.i. and then chased. By 30 min into the chase period, the amount of F_0 was greatly decreased, and by 45 min it was not detectable, but F_1 had appeared, suggesting a precursor-product relationship. The other cleavage product, F_2, was present in the dye front on this gel but is resolved in gels shown below (Fig. 8 and 9). In contrast to the rapid chasing of F_0 into F_1 and F_2, the M protein did not decrease until 3 h, and no other proteins were chased out in up to 5 h. The decrease in M is consistent with the hypothesis that the synthesis of this polypeptide represents a rate-limiting step in the matura-
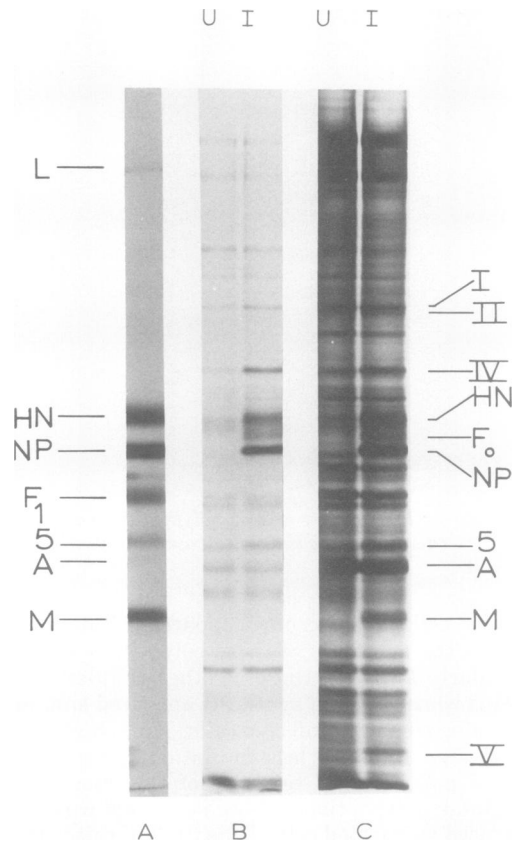

FIG. 5. Effect of high salt concentration on polypeptide synthesis in SV5-infected MDBK cells. (A) Polypeptides of SV5 virions grown in MDBK cells and labeled with [³H]leucine. (B) Polypeptides of infected cells labeled for 60 min at 14 h p.i. with ³H-amino acids in the presence of 0.1 M NaCl. (C) Polypeptides of infected cells labeled for 60 min at 14 h p.i. with ³H-amino acids in isotonic medium. All samples were subjected to electrophoresis on the same slab gel. Fluorography was performed as described in the text.
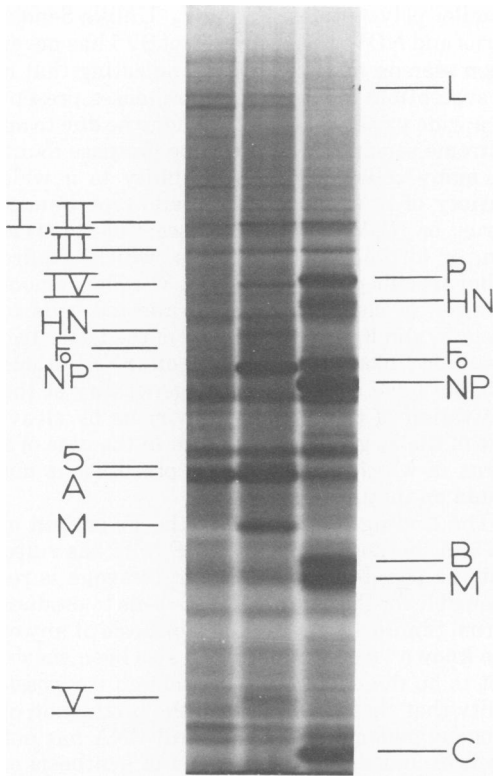
FIG. 6. *Comparison of polypeptides synthesized in SV5- and Sendai virus-infected primary CEF. At 18 h p.i. the cells were labeled with [^{35}S]methionine for 30 min, and cell lysates were then prepared for electrophoresis and autoradiograhy. Left lane, Uninfected cells; middle lane, SV5-infected cells; right lane, Sendai virus-infected cells.*

tion of enveloped viruses (21, 22). There is a slight alteration in the mobility of HN as F_0 disappears, which could represent processing, such as changes in the carbohydrate portion of the glycoprotein. Although proteolytic cleavage involving a significant portion of HN does not appear to occur, removal of a small peptide cannot be excluded.

Glycoprotein synthesis. To obtain further evidence that the transient polypeptide, F_0, is the precursor to F_1 and F_2, infected cells were labeled with [^{3}H]glucosamine. Proteins HN, F_1, and F_2 were labeled, as well as F_0 (Fig. 8). To establish the precursor-product relationship, pulse-chase experiments were performed using [^{3}H]glucosamine. When F_0 diminished, F_1 and F_2 appeared (Fig. 9, left). This is shown more clearly in the right panel of Fig. 9, in which the samples were subjected to electrophoresis in a 20% gel; both F_1 and F_2 are clearly resolved. Although it was not possible to detect a decrease in F_0 since it migrated with HN in this

20% gel, it is evident that F_1 and F_2 are both appearing in the chase. Thus, the experiments shown in Fig. 8 and 9 strongly suggest that F_0 is processed to yield F_1 and F_2. These results, together with the previous findings that F_1 and F_2 are disulfide linked on SV5 virions (38), establish that SV5-infected cells contain an F_0 protein that is proteolytically cleaved to yield two polypeptide chains in a manner analogous to Sendai virus and NDV.

DISCUSSION

The use of high-resolution polyacrylamide gel electrophoresis, autoradiography, and fluorography (20, 21) has made it possible to study SV5 protein synthesis in the presence of ongoing cellular synthesis. The use of several different cell types has made it possible to identify all the known viral proteins and has also revealed several proteins that are not found in the virion but whose synthesis is enhanced in infected cells. By 4 to 6 h p.i. the most abundant viral polypeptide, NP, was seen, and by 12 to 14 h all
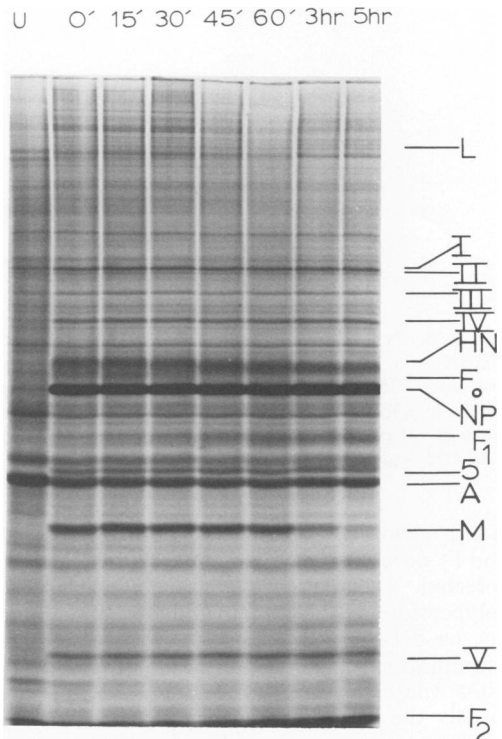

FIG. 7. *Pulse-chase experiment in SV5-infected primary CEF cells. Cells were pulsed for 60 min with [^{35}S]methionine at 17 h p.i. After the pulse, the radioactive medium was removed, the cells were washed three times with PBS, and REM was added. At the times indicated, the cells were prepared for electrophoresis and autoradiography.*
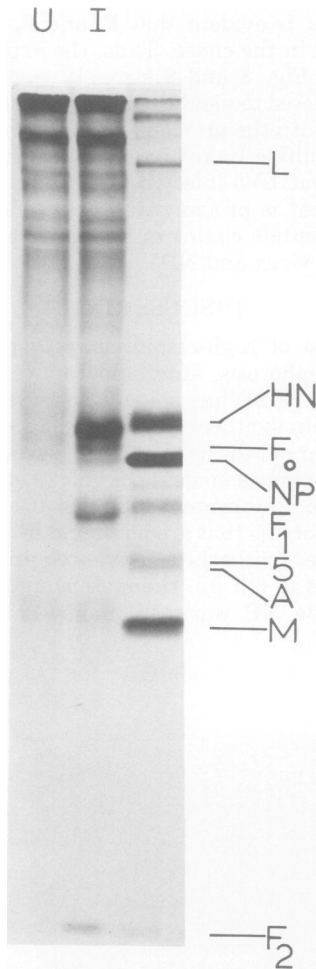
FIG. 8. *Synthesis of glycoproteins in SV5-infected MDBK cells. Cells were labeled for 2 h with [³H]glucosamine (25 µCi/ml) at 17 h p.i. and processed for electrophoresis and fluorography as described in the text. Marker virions (right lane) were labeled with [³H]leucine.*

of the viral structural polypeptides except F_1 and F_2 were present in amounts sufficient to be detected. Although the amount of each viral polypeptide synthesized in infected cells could not be determined precisely by this method, examination of many autoradiographs indicates that they are synthesized in approximately the same unequal proportions as they are found in virions, which suggests that there is control of viral polypeptide synthesis. Similar findings have been reported for NDV (12) and Sendai virus (21, 29).

In pulse-chase experiments a precursor glycoprotein, F_0, has been identified in SV5-infected cells; this appears to be converted to two

smaller polypeptides, F_1 and F_2. Unlike Sendai virus and NDV, the F_0 protein of SV5 has never been seen on mature virions, indicating that it is susceptible to cleavage by proteases present in a wide variety of cells. This may be due to an extreme sensitivity to the same protease found in many cells, or to susceptibility to a wide variety of proteases, unlike wild-type Sendai virus, or NDV, which are susceptible to trypsin, or to the Sendai mutants, which require other specific proteases (27, 35, 37). The demonstration of cleavage of F_0 in infected cells to yield F_1 and F_2 and the finding of the latter two disulfide-linked polypeptides on SV5 virions (33, 38) have established the generality of the activation of paramyxovirus virions by cleavage of the F_0 glycoprotein, even in the case of a virus in which the uncleaved precursor is not found on mature virions.

The finding of cleavage of the F_0 protein of SV5 in the nonpermissive CEF cells has ruled out the possibility that lack of cleavage is responsible for the failure of these cells to produce virus. Similarly, the lack of synthesis of any of the known virion proteins has also been shown not to be the explanation. Although the possibility that there is a block in the production of progeny genome strands of viral RNA has not been excluded, the high levels of synthesis of virion polypeptides in CEP cells suggest that the defect lies at some later step in the assembly process, e.g., in the proper sequence of events at the cell membrane. Further studies are required to explain the failure of production of enveloped viruses from cells in which the known viral proteins are synthesized.

In two avirulent strains of NDV, but not in other strains, Nagai and co-workers (27) demonstrated the existence of a precursor (HN_0) to the viral hemagglutinin-neuraminidase glycoprotein (HN). We have attempted to detect such a precursor in SV5-infected cells, using a variety of experimental conditions, including short pulses with [³⁵S]methionine, high salt concentrations to reduce host cell protein synthesis, and zinc ions, which have been shown in other systems to inhibit cleavage of viral proteins (3, 4, 19). However, we have been unable to detect such a precursor or to detect the synthesis of any other large unstable proteins in infected cells. Similarly, attempts to find an HN_0 precursor in Sendai virus-infected cells have not been successful (21; Lamb and Choppin, unpublished data). Thus, the question remains open of whether synthesis of an HN_0 protein is limited to a few strains, such as the two avirulent strains of NDV, or is a general feature of paramyxoviruses; however, if the latter is the case, it has escaped detection in exten-
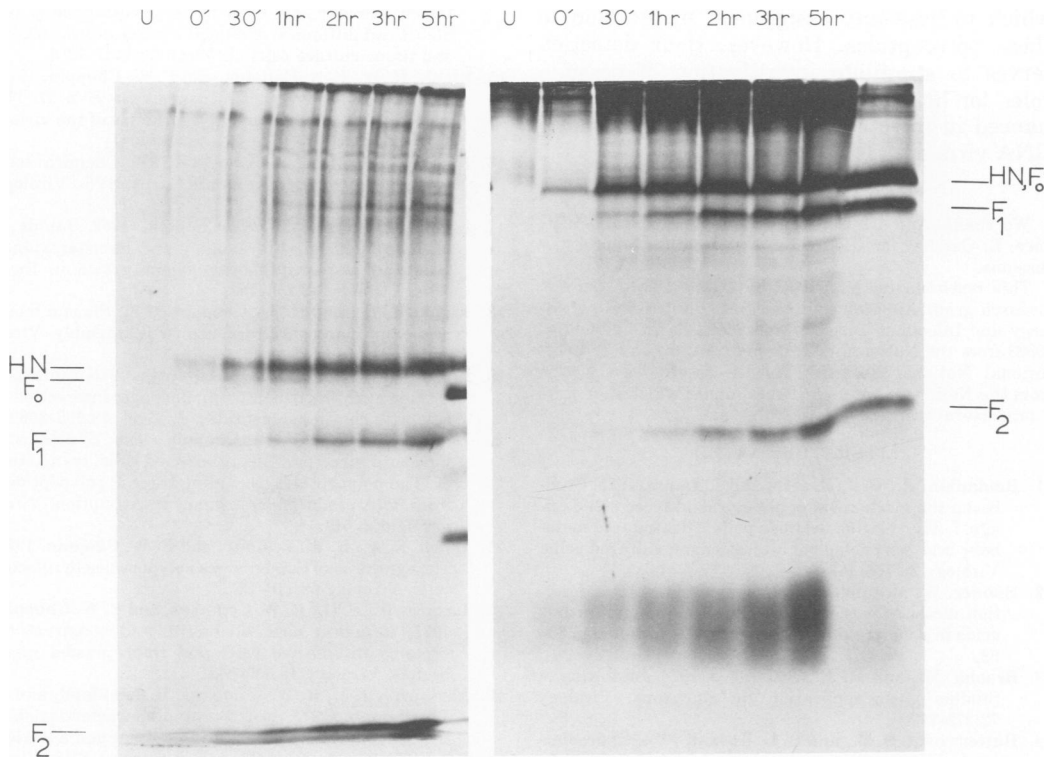
FIG. 9. *Pulse-chase of glycoproteins in SV5-infected MDBK cells. Cells were pulsed for 60 min at 17 h p.i. with [³H]glucosamine (25 µCi/ml) and then washed three times with PBS, and REM was added. At the times indicated the cells were processed as described for electrophoresis on a 10% gel (left) with [³H]leucine-labeled marker virus in the right lane, or on a 20% gel (right) with [³H]glucosamine-labeled marker virus in the right lane.*

sive experiments with SV5 and Sendai virus.

An interesting aspect of the present studies is the observations relating to the polypeptides whose synthesis is stimulated in infected cells but which are not found in the virion. One of these, V (molecular weight ~24,000), does not appear to correspond to a polypeptide found in uninfected cells, and thus may represent a nonstructural protein analogous to the 22,000-dalton, presumed nonstructural polypeptide C synthesized in Sendai virus-infected cells (21). The function of these proteins is at present unknown. In addition to protein V, there are other polypeptides in both SV5- and Sendai virus-infected cells (polypeptides I through IV) whose origins and functions are not certain. However, the available evidence suggests that these are host polypeptides whose synthesis may be enhanced upon infection. This conclusion is based on the finding that there are polypeptides with similar electrophoretic mobilities in uninfected cells, that the number detected depends on the cell type, e.g., three in MDBK cells and four in CEF cells, and that the synthesis of four similar

polypeptides appears to be enhanced in cells infected with Sendai virus. When a high salt concentration was used to selectively decrease the synthesis of host cell proteins, the synthesis of most cellular polypeptides was greatly diminished relative to SV5 proteins; however, synthesis of polypeptide IV, and, to a lesser extent, I, was not inhibited as much as other host cell polypeptides. This suggests that these polypeptides are translated more efficiently in infected cells than in uninfected cells and raises the possibility that they may play some role in the replication of paramyxoviruses, a concept supported by the finding of enhanced synthesis of apparently host polypeptides in Sendai virus-infected cells (Fig. 6). Whether these proteins are significant in virus infection, or, alternatively, their apparent increased synthesis is a nonspecific effect following infection, remains to be determined. There are possible roles that could be envisaged for host proteins in paramyxovirus infection, e.g., as a component of an enzyme involved in the replication of genome RNA, but there is no evidence at present on

which to base any assignment of function to these polypeptides. However, their detection serves to stimulate investigation of possible roles for host proteins whose synthesis is enhanced in infection by these nontransforming RNA viruses.

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