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Characterization of V Protein in Measles Virus-Infected Cells

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An edited mRNA transcribed from the phosphoprotein (P) gene of measles virus (MV) has been predicted to encode a cysteine-rich protein designated V. This mRNA contains a single additional nontemplated G residue which permits access to an additional protein-coding reading frame. Such an edited P gene-specific mRNA has been detected in MV-infected cells, but no corresponding protein has yet been identified in vivo. We report the use of antisera directed against synthetic peptides corresponding to five different regions of the predicted MV V protein amino acid sequence to analyse MV-specific proteins synthesized in vivo and in vitro. The MV V protein (40 kDa) was detected in MV-infected cells in a diffuse cytoplasmic distribution, a predominant subcellular localization distinct from that of virus nucleocapsids. The protein was found to be phosphorylated and to be maximally synthesized at 16 h postinfection, when MV-specific structural protein synthesis was also maximal. Antiserum directed against a peptide (PV2) corresponding to amino acids 65 to 87 of the V protein amino acid recognized the P protein but not the V protein, indicating that the P and V proteins may be folded differently at or near this region so that the PV2 sequence is in an exposed position at the surface of the P protein but not at the surface of the V protein.

Measles, a highly contagious acute viral disease of childhood, is caused by measles virus (MV), a member of the morbillivirus subgroup of the paramyxovirus family of negative-stranded RNA viruses. In rare cases, persistent infection with MV results in subacute sclerosing panencephalitis, a uniformly fatal central nervous system disease (25). Fortunately, infection with MV can be prevented by administration of a live attenuated virus vaccine (7, 8).

The MV genome is a nonsegmented single-stranded negative-sense RNA with an estimated M_r of 4,500,000 (3, 14). The complete genomic sequence of MV has now been determined. It contains approximately 15,900 nucleotides, the exact number depending on the virus strain (17). Six structural proteins have their genes sequentially arranged along the MV genome: the nucleoprotein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the hemagglutinin (HA), and the large or polymerase protein (L). In addition, a small nonstructural polypeptide (C) has been identified in MV-infected cells (4).

It has recently been demonstrated that multiple different species of mRNA transcripts can be derived from P genes of a number of paramyxoviruses (6, 12, 19, 23, 24, 26, 27). Although the process has been termed a form of RNA editing, these different mRNA species appear to result from the cotranscriptional addition of nontemplated G residues at at least one specific location (27). This can cause changes in the protein-coding reading frame in some mRNAs, leading to the synthesis of at least two different proteins coterminal at their amino termini: the P protein and a cysteine-rich protein termed V. Two slightly different coding strategies exist among paramyxoviruses for the expression of P and V proteins. The MV and Sendai virus P proteins are encoded by mRNAs faithful to the genomic sequence, while the synthesis of V protein requires an additional nontemplated G residue within its mRNA sequence (6, 27). On the other hand, during infection with simian virus 5 (SV5), mumps virus, or parainfluenza virus type 2 or type 4, mRNAs faithful to the original genomic sequence encode V while mRNAs containing nontemplated G residues encode P (12, 18, 19, 26). The relative frequency of mRNAs containing such G insertions has been estimated to be 50% for MV (6), 45% for SV5 (26), 31% for Sendai virus (27), and 40 to 50% for mumps virus (19, 24).

An edited mRNA derived from the MV P gene has been identified in vivo (6) by cDNA cloning, cDNA and RNA sequencing, and in vitro translation. The V protein-specific mRNA was found to contain an extra G nucleotide inserted at a site comprising three genomically encoded G residues corresponding to positions 752 to 754 of the originally published P gene sequence (4). The edited mRNA was predicted to encode a V protein containing 299 amino acids, and a corresponding protein band could be seen after in vitro transcription and translation of the corresponding cDNA clone. The authors did not, however, provide any direct evidence that a protein corresponding to the V coding region was present in MV-infected cells.

In this article we report the generation and characterization of antisera directed against a series of synthetic peptides corresponding to different regions of the predicted amino acid sequence of the MV V protein. We have used these antisera as probes to definitely identify and to characterize the in vivo pattern of V protein expression in MV-infected cells.

MATERIALS AND METHODS

Cells and viruses. Vero cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% fetal bovine serum. The Edmonston strain of MV (American Tissue Type Collection) was propagated as previously described (2).

Generation of antisera against synthetic peptides. The synthesis of five peptides corresponding to different regions of the MV V protein (Table 1) was commissioned from Immunodynamics (La Jolla, Calif.). Peptides were coupled to

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TABLE 1. Amino acid sequences of synthetic peptides used to generate monospecific and polyspecific rabbit antisera against the MV P and V proteins

Peptide	Amino acid sequence	Location within V protein[a]
PV1	IRALKAEPIGSLAIEEAMAA	16–35
PV2	LSAIGSTEGGAPRIRGQGPGESD	65–87
PV3	DIGEPDTEGYAITDRGSAPIS	154–174
V1	SKVTLGTIRAR	256–266
V2	RTDTGVDTRIWYHNLPEIPE	280–299

[a] Numbering reflects the sequence of MV V protein assuming addition of a single nontemplated G residue to the three templated G residues at positions 252 to 254 of the published MV P gene sequence (4) as subsequently corrected (6).

keyhole limpet hemocyanin (KLH) or bovine serum albumen (BSA) by addition of an amino-terminal cysteine residue before treatment with *m*maleimidobenzoyl-*N*-hydroxysuccinimide ester. Peptide V2 (corresponding to the exact carboxyl terminus of V) was left with a free carboxyl group at its carboxyl terminus, while all other peptides were synthesized with carboxyl-terminal carbamino groups.

Duplicate New Zealand White rabbits were initially immunized with peptide-KLH conjugate in Freund's complete adjuvant. Approximately 0.2 mg of peptide conjugate was injected subcutaneously at each of five sites along the back. Rabbits were subsequently reimmunized a total of four times at 4- to 6-week intervals in a similar manner with peptide-conjugate in Freund's incomplete adjuvant. All resulting antisera were tested for their ability to react with the peptides used in immunization by enzyme-linked immunosorbent assays (ELISAs) with peptide-BSA conjugates as the antigen. Preimmune sera were used as negative controls.

Construction of plasmids for in vitro transcription of mRNAs specific to the P and V proteins. The MV V transcript differs from the MV P transcript by the addition of a nontemplated G residue at the stretch of three templated G residues at positions 752 to 754. We had previously described cloning of a cDNA of the MV P coding region into the *Pst*I site of pBR322, creating pMV-P (2). We have now used a site-specific mutagenesis protocol to covert this into a copy of the V protein-coding region. The P coding region consisted of 1,565 nucleotides bounded by a 5′ *Xho*I site and a 3′ *Hin*cII site. The plasmid containing this coding region was digested with *Sac*I to release a DNA fragment 765 nucleotides in length that contained the G insertion site. The fragment was then ligated into the *Sac*I site of M13mp19 (Bio-Rad), and oligonucleotide-directed site-specific mutagenesis was performed according to the instructions of the supplier. The primer used for mutagenesis (Regional DNA Synthesis Laboratory, University of Calgary, Calgary, Canada) had the sequence 5′-dCCATTAAAAAGGGGCACAG AG-3′, where the underlined G was the additional nontemplated nucleotide. Confirmation of successful mutagenesis was obtained by dideoxy chain termination sequencing of alkali-denatured plasmid DNA performed with deoxyadenosine-5′-[^{35}S]thiotriphosphate (500 Ci/mmol; Amersham Corp.) and T7 DNA polymerase (Sequenase; United States Biochemical Corp.) according to the protocol of the suppliers. The primer used for sequencing was 5′-dCTTTCCGA AGCTTGG-3′ (Regional DNA Synthesis Laboratory, University of Calgary). The mutated *Sac*I fragment was inserted into the *Sac*I site of pMV-P to create plasmid pMV-V, containing the V coding sequence. Plasmids pMV-P and pMV-V were digested with *Xho*I and *Hin*cII to release the P and V coding sequences, which were then separately inserted into an SP6/T7 vector (Amersham) to create pAM18MV-P and pAM19MV-V, respectively.

In vitro transcription and translation. In vitro transcription in the presence of the cap analog diguanosine triphosphate [$m^7G(5')ppp(5')G_m$; Pharmacia] was performed with SP6 polymerase according to the instructions of the supplier (Pharmacia). The resulting RNA transcripts were translated in micrococcal nuclease-treated rabbit reticulocyte lysate according to the instructions of the supplier (Promega Corp.).

In vivo radiolabeling of infected-cell proteins. Confluent monolayers of Vero cells were infected with MV at a multiplicity of infection of 10 PFU per cell. Cells were pulse-labeled as described previously (2) with either Tran[^{35}S]label (ICN Radiochemicals), [^{35}S]cysteine, [^{35}S]methionine, or $^{32}P_i$ (Amersham Corp.) in minimum essential medium deficient in methionine, cysteine, or phosphate, depending on the labeled substance added.

Analysis of MV-specific proteins. In vivo- and in vitro-synthesized proteins were incubated in RIPA buffer containing 0.1% BSA and the appropriate antiserum for 2 h at 4°C. For immunoprecipitation of in vivo-synthesized proteins, 100 μl of cell lysate was mixed with 5 μl of antiserum. For immunoprecipitation of in vitro-synthesized proteins, 5 μl of the 50-μl total translation reaction was mixed with 5 μl of antiserum. Protein A-Sepharose beads (Pharmacia) were subsequently added to each mixture, and the mixtures were then incubated for 1 h at 4°C. Immune complexes were washed three times with RIPA buffer containing 1% BSA and twice with RIPA buffer without BSA. Then 35 μl of protein lysis buffer was added, incubated at 37°C for 10 min, heated to 95°C for 3 min, and analyzed by 10% discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (13).

Indirect immunofluorescence. Vero cells were grown to 40% confluence on microscope coverslips before infection with MV. Uninfected Vero cells were used as controls. After 16 h, cells were washed with phosphate-buffered saline (PBS) and fixed by treatment with 2.5% paraformaldehyde for 20 min. Cells were permeabilized by treatment with 0.5% Triton X-100 in PBS for 10 min. Following three washes with PBS, cells were treated with normal goat serum (Cedarlane) for 30 min and then with primary antiserum for 1 h. A control was done without addition of primary antibody for both infected and uninfected cells. Cells were then washed three times with PBS and treated with goat anti-rabbit immunoglobulin G (IgG) coupled to fluorescein isothiocyanate (FITC) (Cedarlane) for 30 min. Cells were washed again with PBS before inversion over a drop of glycerol containing Citofluor (Amersham) on glass slides for photography.

RESULTS

Preparation of antibodies directed against synthetic peptides. In order to study the expression of V protein in MV-infected cells, five peptide sequences were chosen from the predicted amino acid sequence of the V protein for the production of antipeptide antibodies (Table 1). Peptides were chosen to maximize their hydrophilicity while also optimizing location and secondary-structure predictions. Three of the peptides synthesized (PV1, PV2, and PV3) map to the amino-coterminal region of the P and V proteins, and antibodies directed against these peptides were expected to

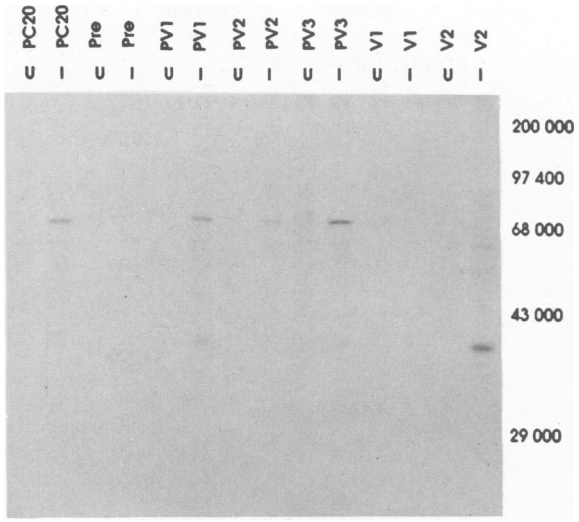

FIG. 1. Analysis of [^{35}S]methionine-labeled polypeptides from uninfected (U) Vero cells as well as from Vero cells 16 h after infection with MV (I). Cell lysates were immunoprecipitated with preimmune serum (Pre) or peptide antiserum PC20, PV1, PV2, PV3, V1, or V2. Samples were analyzed on a 10% SDS–polyacrylamide gel. Positions of molecular weight markers (not shown) are indicated on the right.

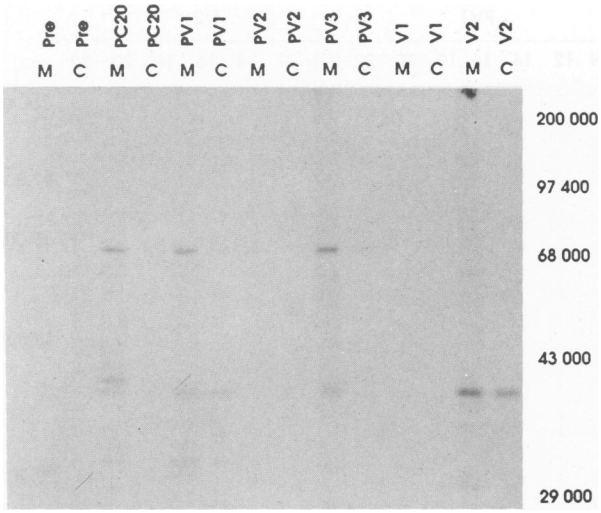

FIG. 2. Analysis of polypeptides from Vero cells 16 h after infection with MV following differential radiolabeling with [^{35}S]methionine (M) or [^{35}S]cysteine (C) and immunoprecipitation with preimmune serum (Pre) or peptide antiserum PC20, PV1, PV2, PV3, V1, or V2. Samples were analyzed on a 10% SDS–polyacrylamide gel. Positions of molecular weight markers (not shown) are indicated on the right.

be able to recognize both P and V. The remaining two peptides (V1 and V2) map to the unique carboxyl terminus of the V protein, and antibodies directed against these peptides were expected to be able to recognize the V protein alone.

After synthesis, the peptides were conjugated with KLH, and each was used to immunize two New Zealand White rabbits. The titers of the resulting antibody response were determined by ELISA with preimmune serum as a negative control. After a series of five immunizations for each rabbit, all antisera had titers of at least 10^5 except that directed against V1. This last antiserum reached a titer of only 5×10^4. Nevertheless, the antibody titer of the antiserum directed against the V1 peptide would normally be considered adequate for use in immunoprecipitation. Preimmune sera from all 10 rabbits were pooled and then used as a negative control. Additionally, peptide antiserum PC20 (generously provided by C. D. Richardson), directed against a carboxy-terminal region unique to the P protein (20) and thus not expressed in the V protein, was also used as a control.

Detection of V protein in MV-infected cells. To determine whether the MV V protein was indeed synthesized in vivo, [^{35}S]methionine-labeled proteins from MV-infected Vero cells were immunoprecipitated with our different antisera and analyzed by SDS-PAGE. The MV P protein has a predicted size of approximately 72 kDa (17), while the V protein has been predicted to be approximately 40 kDa (6). No proteins were detected when uninfected-cell lysates were immunoprecipitated with any of our peptide antisera or when infected-cell lysates were immunoprecipitated with preimmune serum (Fig. 1). However, two polypeptides with migrations corresponding to 70 and 40 kDa were detected in MV-infected cell lysates in patterns corresponding to the predicted specificity of the different peptide antisera and were considered likely to be the P and V proteins. Evidence in support of such identification is that (i) the 70-kDa polypeptide migrated at the same position as the P immunoprecipitated by the PC20 antiserum (Fig. 1, lane 2), (ii) the 70-kDa polypeptide was recognized by antisera predicted to be specific to both the P and V proteins (PV1, PV2, and PV3) but not by antisera predicted to be specific to the V protein alone (V1 and V2), and (iii) the 40-kDa polypeptide was recognized by the V-only specific antiserum V2 as well as by antisera specific to both P and V (PV1 and PV3) but not by the P-only specific antiserum PC20.

Despite multiple attempts at immunoprecipitation and autoradiographs of much longer exposure and greater intensity than those shown, neither the V1 nor the PV2 antisera could detect a band corresponding to the V protein. However, the V1 peptide was predicted to be the most hydrophobic of our synthetic peptides. The region of amino acid sequence unique to V encoded after the G insertion site contains only 62 amino acids. The V1 peptide sequence seemed, after that of V2, to be the most favorable for antibody recognition based on secondary-structure predictions. Such predictions do not, however, guarantee antibody recognition of the native protein. The PV2 antiserum was able to immunoprecipitate the 70-kDa polypeptide but not the 40-kDa polypeptide, although the sequence of the peptide was derived from a portion of the P/V amino-coterminal region. This may indicate that the P and V proteins are folded differently at or near this region so that the PV2 sequence is in an exposed position at the surface of the P protein but not at the surface of the V protein.

Immunoprecipitation of differentially radiolabeled proteins from MV-infected cells. In order to provide more evidence that the 70- and 40-kDa polypeptides indeed represented, respectively, the P and V proteins, MV-infected cells were separately and differentially radiolabeled with either [^{35}S]methionine or [^{35}S]cysteine, immunoprecipitated with our different antisera, and then analyzed by SDS-PAGE (Fig. 2). The MV P protein contains 11 methionine and 6 cysteine residues, whereas the V protein is predicted to contain 5 methionine and 11 cysteine residues. It can be seen in the control lanes, where the PC20 antiserum was used,

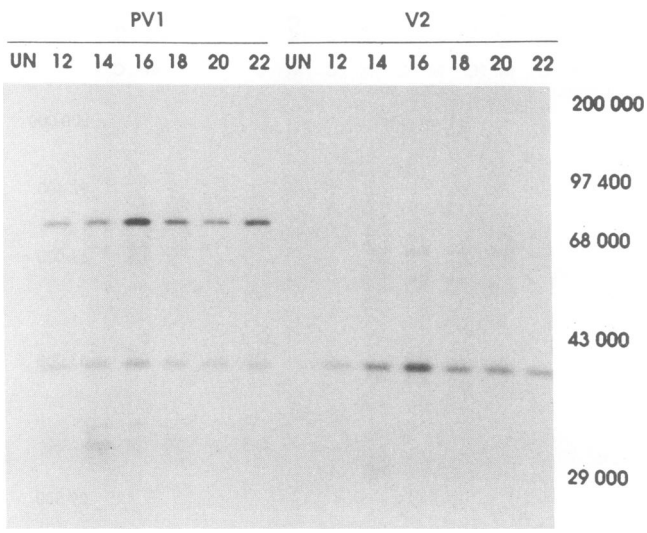

FIG. 3. P and V protein synthesis late in infection. Vero cells were labeled with [^{35}S]methionine at the times noted (in hours) following infection with MV and immunoprecipitated with peptide antiserum PV1 or V2. Samples were analyzed on a 10% SDS–polyacrylamide gel. Positions of molecular weight markers (not shown) are indicated on the right. Uninfected Vero cells (UN) were used as a control.

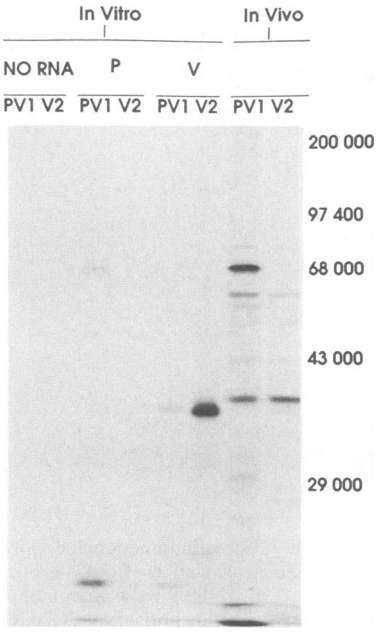

FIG. 4. Comparison of P and V proteins synthesized in vivo and in vitro. [^{35}S]methionine-labeled polypeptides from Vero cells 16 h after infection with MV and [^{35}S]methionine-labeled polypeptides resulting from in vitro translation in rabbit reticulocyte lysate of RNA transcribed in vitro from linearized DNA from plasmids pAM18MV-P (P) and pAM19MV-V (V) were immunoprecipitated with peptide antiserum PV1 or V2 and analyzed on a 10% SDS–polyacrylamide gel. Positions of molecular weight markers (not shown) are indicated on the right.

that the efficiency of cysteine labeling was not as great as that of methionine labeling. While a band representing the P protein labeled with methionine was clearly visible, no equivalent band representing P protein labeled with cysteine could be clearly identified. This could be due to a number of factors, including differential stability of the radiochemicals or their differential transport into cells, as well as different cellular amino acid pool sizes. The P protein was also more readily labeled with methionine than with cysteine when immunoprecipitated with the PV1, PV2, or PV3 antiserum (Fig. 2). Radiolabeling of the 40-kDa polypeptide with cysteine, on the other hand, was relatively easier to accomplish (Fig. 2, lanes immunoprecipitated with PV1, PV3, and V2 antisera). The data therefore support the identification of these two polypeptides as the MV P and V proteins.

Time course of V protein synthesis during MV infection. To determine the time course of V protein synthesis in vivo, cells were labeled with [^{35}S]methionine at different times following MV infection. Labeled proteins were immunoprecipitated with PV1 or V2 antiserum and analyzed by SDS-PAGE. Neither the P nor the V protein was detectable before 8 to 10 h postinfection (data not shown). Figure 3 shows levels of synthesis of the P and V proteins at later times after infection. Maximal synthesis occurred at 16 h after infection, at which time approximately 80 to 90% of the cells exhibited cell fusion. The time of maximal in vivo synthesis of MV structural proteins for the virus stock used had also previously been determined to be 14 to 18 h after infection (data not shown).

Analysis of polypeptides synthesized in vitro. To confirm the specificity of the peptide antisera for the P and V proteins, the antisera were tested for their ability to immunoprecipitate in vitro-synthesized P and V. Two plasmids, pAM18MV-P and pAM19MV-V, containing, respectively, the P and V coding regions, were constructed so as to be able to direct the in vitro transcription of RNAs specific to the P and V proteins. Rabbit reticulocyte lysate was subsequently used for in vitro translation reactions with the in vitro-transcribed RNAs.

Polypeptides of 70 and 40 kDa were immunoprecipitated from in vitro translation reactions by peptide antisera and were not present in control in vitro translation reactions to which RNA was not added (Fig. 4). In vitro-synthesized P protein was consistently found to be less abundant than V protein. This is probably the result of the inherently lower efficiency of the reticulocyte lysate system in the translation of higher-molecular-weight species but may also reflect differential in vitro stability of the proteins. Proteins were also immunoprecipitated from MV-infected cell lysates to compare the migration of in vivo- and in vitro-synthesized proteins. Polypeptides synthesized in vitro corresponding to both P and V migrated slightly faster than the corresponding proteins synthesized in vivo. This probably indicates that these proteins exhibit different degrees of posttranslational modification in vivo and in vitro.

Proteins synthesized in vitro were differentially labeled with either [^{35}S]methionine or [^{35}S]cysteine of equivalent total and specific activity and analyzed by SDS-PAGE (Fig. 5). Under these conditions, the intensity of labeling of the 70-kDa polypeptide was at least as great with methionine as with cysteine. The intensity of labeling of the 40-kDa polypeptide was, however, significantly greater with cysteine than with methionine. This pattern of labeling is consistent with the different expected ratios of methionine to cysteine in the P and V proteins. Since stability, cell transport, cellular amino acid pool size, etc., are not factors in vitro, these results strongly confirm the identity of these proteins.

Analysis of ^{32}P-labeled P and V proteins. In order to

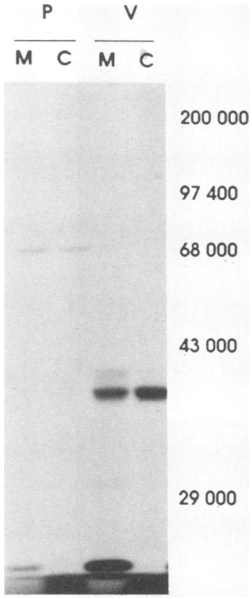

FIG. 5. Analysis of in vitro-synthesized P and V proteins differentially radiolabeled with either [^{35}S]methionine (M) or [^{35}S]cysteine (C) at equivalent total and specific activities. Linearized DNA from plasmids pAM18MV-P (P) and pAM19MV-V (V) was transcribed in vitro, and the resulting mRNAs were translated in vitro in a rabbit reticulocyte lysate. Polypeptides were analyzed on a 10% SDS–polyacrylamide gel. Positions of molecular weight markers (not shown) are indicated on the right.

determine whether the V protein was phosphorylated, MV-infected cells were pulse-labeled with $^{32}P_i$ at 16 h after infection, immunoprecipitated, and analyzed by SDS-PAGE (Fig. 6). The P protein has previously been shown to be highly phosphorylated (10), and our results confirm this. It

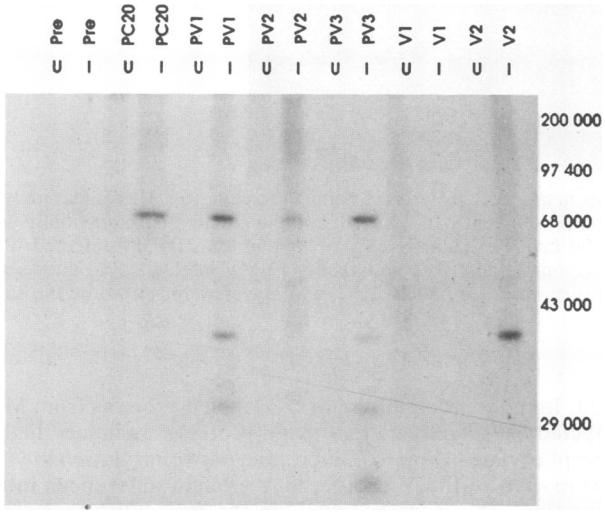

FIG. 6. Analysis of polypeptides from uninfected Vero cells (U) and from Vero cells 16 h after infection with MV (I). Cells were pulse-labeled for 1 h with $^{32}P_i$. Cell lysates were immunoprecipitated with preimmune serum (Pre) or peptide antiserum PC20, PV1, PV2, PV3, V1, or V2. Samples were analyzed on a 10% SDS–polyacrylamide gel. Positions of molecular weight markers (not shown) are indicated on the right.

can clearly be seen that the V protein is also phosphorylated in vivo.

Subcellular localization of the MV P and V proteins. In order to determine the exact cellular localization of the P and V proteins in MV-infected cells, indirect immunofluorescence analysis was performed. Uninfected cells exhibited no fluorescence with any of the antisera (Fig. 7). Similarly, no fluorescence was observed in uninfected or MV-infected cells that were not treated with primary antibody (data not shown). The PV2 antiserum (specific only to P protein when used for immunoprecipitation) resulted in specific fluorescence in small discrete cytoplasmic inclusions. This result is similar to that described previously (4) for a peptide antiserum against a carboxy-terminal region of P which is not present in V. Hence, the specificity of immunofluorescence resulting from use of the PV2 antiserum does seem to represent P alone. The V2 antiserum (specific only to the V protein when used for immunoprecipitation) resulted in a different pattern, a diffuse cytoplasmic fluorescence. The PV1 antiserum (specific to both the P and V proteins when used for immunoprecipitation) gave a result appearing to combine the patterns obtained with the PV2 and V2 antisera.

DISCUSSION

Since Thomas et al. (26) reported that the SV5 P protein was expressed from an edited mRNA transcript, a number of groups have reported similar RNA editing in transcripts arising from the P genes of a number of other paramyxoviruses. The discovery of this type of RNA editing came together with the discovery of a previously unknown paramyxovirus-encoded protein which has come to be designated the V protein. Analogous V proteins have now been detected in cells infected with a number of paramyxoviruses. P protein-specific monoclonal antibodies were used for the detection of V protein in SV5- and parainfluenza virus type 2-infected cells (23, 26). Antisera directed against synthetic peptides were similarly used to detect V in mumps virus-infected cells (19, 24). An edited P gene-specific mRNA has been detected in MV- and Sendai virus-infected cells, but there has been no direct evidence for the in vivo existence of either a Sendai virus- or MV-specific V protein. Synthetic peptide-specific antisera directed against the MV V protein were produced in the present study for this purpose. With these antisera, a cysteine-rich polypeptide was detected in MV-infected cells by immunoprecipitation and immunofluorescence analysis. The V protein was found to migrate as a 40-kDa polypeptide. Rima et al. (21) reported the detection of an MV-specific 40-kDa protein possibly related to the P protein that comigrated with the MV M protein and used partial protease digestion to suggest that it was not simply a breakdown product of P. We now report the definite identification of the MV V protein, and we have also found it to comigrate with the M protein (data not shown). This probably explains previous difficulties in recognizing and identifying the MV V protein. No strong signal at 40 kDa had previously been detected in MV-infected cells after immunoprecipitation with antibodies directed against the exact amino terminus of the P protein (4). This may be because the carboxyl terminus of paramyxovirus P proteins is, in general, more immunogenic than the rest of the molecule (28).

The gel migration of P and V proteins immunoprecipitated from MV-infected cells was compared with that of those immunoprecipitated from in vitro translation reactions. The in vitro-synthesized P and V proteins were found to migrate slightly faster than those immunoprecipitated from MV-

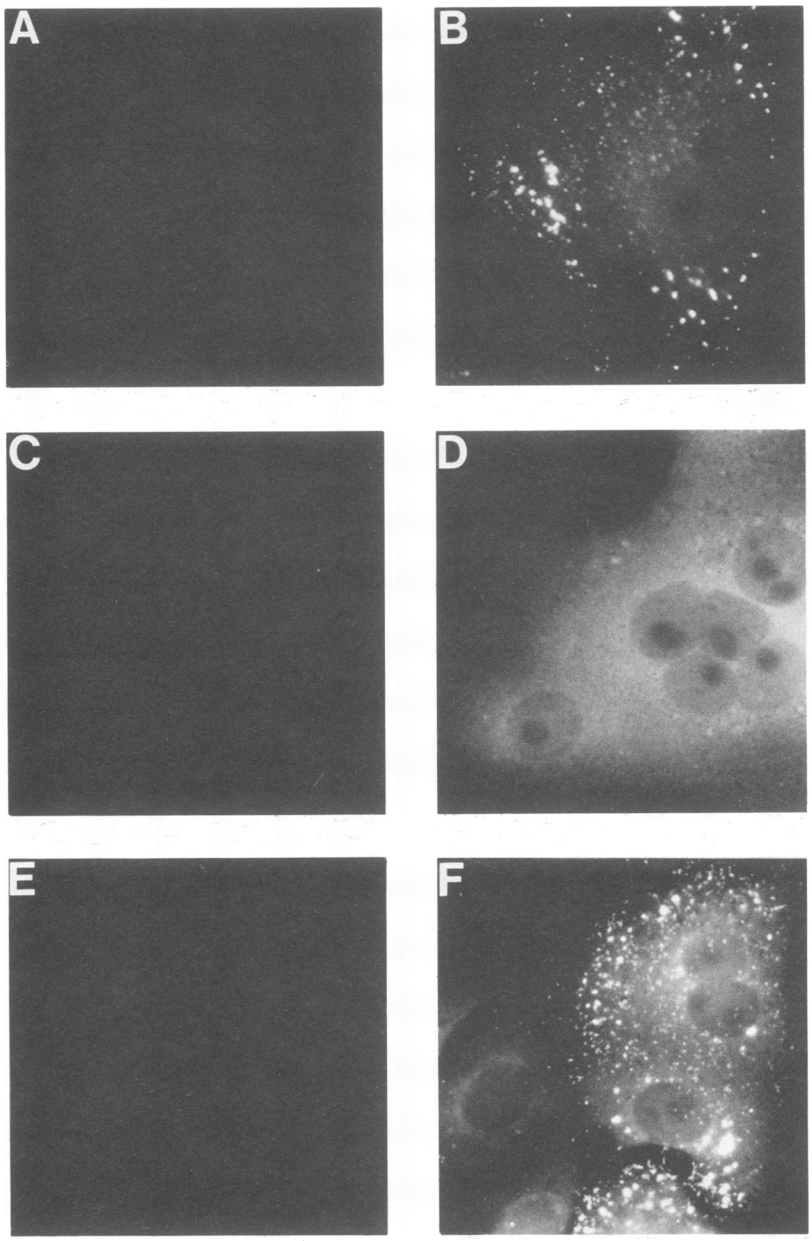

FIG. 7. Indirect immunofluorescence analysis of the MV P and V proteins in uninfected Vero cells and Vero cells 16 h after infection with MV. Cells were fixed in 2.5% paraformaldehyde in PBS, permeabilized with 0.5% Triton X-100 in PBS, and then incubated sequentially with normal goat serum, peptide antiserum, and FITC-conjugated goat anti-rabbit IgG. (A) Uninfected, PV2 antiserum; (B) MV infected, PV2 antiserum; (C) uninfected, V2 antiserum; (D) MV infected, V2 antiserum; (E) uninfected, PV1 antiserum; (F) MV infected, PV1 antiserum. UV photomicrographs taken at ×400 magnification. Photomicrograph exposure times for panels A, C, and E were adjusted to be the same as those for panels B, D, and F.

infected cells (Fig. 4). This probably indicates that different posttranslational modifications occur in vivo and in vitro. Such differences would not be surprising since the rabbit reticulocyte lysate system has been shown to contain a variety of posttranslational processing activities which may lead to gel migration differences. These include proteolysis (15), phosphorylation (9), acetylation (22), and isoprenylation (29). The P protein is known to be phosphorylated, and since the V protein shares the amino-terminal 231 of its 299 amino acids with the amino terminus of the P protein, the possibility of phosphorylation of the V protein was examined. Immunoprecipitation of ^{32}P-labeled proteins from MV-infected cells indicated that the V protein was indeed highly phosphorylated (Fig. 6). Differential phosphorylation in vitro and in vivo of the V protein may explain differences in gel migration. This does not exclude the possibility that other modifications may distinguish the V protein synthesized in vivo from that synthesized in vitro.

Paterson and Lamb (19) have reported that an additional mumps virus-specific protein, designated I, of approximately 19 kDa is encoded by an additional species of edited mRNA transcribed from the virus P gene. In this transcript, four

nontemplated G residues are added instead of the two which give rise to the P mRNA. This results in a shift into yet another reading frame distal to the G insertion point which is distinct from that of either P or V. Takeuchi et al. (24) have reported immunoprecipitation of a mumps virus-specific protein of approximately 24 kDa with antiserum directed against a synthetic peptide corresponding to a part of the amino-coterminal region of P and V. This protein may well be the product of an mRNA to which four nontemplated G residues are added. An analogous protein could potentially be encoded by MV if G residues were added to the insertion point in the P gene transcript to cause a shift into the +1 reading frame relative to that of P (i.e. 2, 5, 8, etc.). Translation of such a message would terminate only five codons after the insertion site. Cattaneo et al. (6) reported that 2 of 19 cDNA clones specific to MV P gene-encoded mRNA species had insertions of three G's, but no clones were obtained which could encode a putative MV I protein. However, this may only reflect examination of an insufficient number or unrepresentative sampling of cDNA clones. We were unable to detect any such putative MV-specific protein with our antisera for immunoprecipitation analysis of MV-infected cell lysates. We were, in some instances, able to detect proteins of low molecular weight, but these did not appear to be additional species of amino-coterminal yet carboxy-dissimilar proteins since they were also immunoprecipitated with the PC20 antiserum, which is directed against a carboxyl-terminal region of the P protein (Fig. 2). The exact nature of these proteins has not yet been fully determined.

The MV V protein is diffusely present in the cytoplasm of MV-infected cells (Fig. 7). The P protein localizes in a distinctly different pattern, in small bright cytoplasmic inclusions. Earlier studies have shown that such cytoplasmic inclusions also contain viral nucleocapsids (11, 16). Since the P protein thus appears to be a marker for nucleocapsids, most MV V protein does not therefore appear to be associated with nucleocapsids during the period of maximal virus protein synthesis. In fact, when MV-infected cells were analyzed with an antiserum able to recognize both P and V, a mixture of both of these distinct fluorescence patterns was seen. This does not, however, exclude the possibility that a very small proportion of V protein may nonetheless be associated with nucleocapsids. A number of investigators have reported that anti-P antibodies are also able to immunoprecipitate the V, NP, and possibly also L proteins of some paramyxoviruses and have taken this to imply an association of V with transcriptional complexes (24, 26). We also found that a small amount of the NP protein could be immunoprecipitated when either P- or V-specific antiserum was used. However, we have previously found that small amounts of the NP could be immunoprecipitated with antiserum directed against the MV hemagglutinin (1). The significance of this phenomenon remains unclear.

The function of the paramyxovirus V protein remains obscure. An open reading frame for V protein is highly conserved among paramyxoviruses, and it thus seems likely that it has an important biological role. Since the cysteine-rich domain in the unique region of paramyxovirus V proteins resembles zinc finger motifs that have been identified as participating in binding with nucleic acids or proteins, it has been suggested that the V protein may be a regulatory factor involved in virus RNA or protein synthesis (6, 26). Delineation of the exact nature of the function of the V protein will involve additional research, likely requiring the ability to produce large amounts of purified protein for functional assays or else the development of virus mutants deficient in the expression of V protein.

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