JOURNAL OF VIROLOGY, Aug. 1990, p. 3627–3634
0022-538X/90/083627-08$02.00/0

Vol. 64, No. 8

Organ Tropism of Sendai Virus in Mice: Proteolytic Activation of the Fusion Glycoprotein in Mouse Organs and Budding Site at the Bronchial Epithelium

M. TASHIRO,[1]* M. YAMAKAWA,[2] K. TOBITA,[1] H.-D. KLENK,[3] R. ROTT,[4] AND J. T. SETO[5]

Department of Virology, Jichi Medical School, Minami-Kawachi, Tochigi-Ken, 329-04,[1] and Department of Pathology, Yamagata University School of Medicine, Yamagata, 990-23,[2] Japan; Institut für Virologie, Philipps-Universität Marburg, D-3550 Marburg,[3] and Institut für Virologie, Justus-Liebig-Universität Giessen, D-6300 Giessen,[4] Federal Republic of Germany; and Department of Microbiology, California State University at Los Angeles, Los Angeles, California 90032-8745[5]

Received 5 October 1989/Accepted 11 April 1990

Wild-type Sendai virus is exclusively pneumotropic in mice, while a host range mutant, F1-R, is pantropic. The latter was attributed to structural changes in the fusion (F) glycoprotein, which was cleaved by ubiquitous proteases present in many organs (M. Tashiro, E. Pritzer, M. A. Khoshnan, M. Yamakawa, K. Kuroda, H.-D. Klenk, R. Rott, and J. T. Seto, Virology 165:577–583, 1988). These studies were extended by investigating, by use of an organ block culture system of mice, whether differences exist in the susceptibility of the lung and the other organs to the viruses and in proteolytic activation of the F protein of the viruses. Block cultures of mouse organs were shown to synthesize the viral polypeptides and to support productive infections by the viruses. These findings ruled out the possibility that pneumotropism of wild-type virus results because only the respiratory organs are susceptible to the virus. Progeny virus of F1-R was produced in the activated form as shown by infectivity assays and proteolytic cleavage of the F protein in the infected organ cultures. On the other hand, much of wild-type virus produced in cultures of organs other than lung remained nonactivated. The findings indicate that the F protein of wild-type virus was poorly activated by ubiquitous proteases which efficiently activated the F protein of F1-R. Thus, the activating protease for wild-type F protein is present only in the respiratory organs. These results, taken together with a comparison of the predicted amino acid substitutions between the viruses, strongly suggest that the different efficiencies among mouse organs in the proteolytic activation of F protein must be the primary determinant for organ tropism of Sendai virus. Additionally, immunoelectron microscopic examination of the mouse bronchus indicated that the budding site of wild-type virus was restricted to the apical domain of the epithelium, whereas budding by F1-R occurred at the apical and basal domains. Bipolar budding was also observed in MDCK monolayers infected with F1-R. The differential budding site at the primary target of infection may be an additional determinant for organ tropism of Sendai virus in mice.

Sendai virus, a prototype of the paramyxovirus family, is exclusively pneumotropic in mice (4, 10, 36, 38), despite the wide distribution of receptors in various organs of mice (11). Posttranslational cleavage of the fusion (F) glycoprotein into F_1 and F_2 subunits is a prerequisite for the virus to express infectivity (7, 8, 28, 29). Therefore, the host range and organ tropism of Sendai virus have been proposed to be primarily attributed to the presence of an activating protease(s) in target tissues and the susceptibility of F protein to the protease(s) (5, 10, 15, 23, 36, 39). The cleavage site of F protein contains a single arginine residue. The protein should be resistant to a ubiquitous cellular protease(s) which preferentially cleaves dibasic or multibasic amino acid sequences (1, 9, 12, 26, 33). Thus, the activating enzyme which cleaves the single arginine residue of F protein should be present only in restricted cell types, such as the chorioallantois of embryonated chicken eggs (22), several primary tissue culture cells (30, 34), or lungs of mice (36), in which cell types virus can undergo multiple cycles of replication. We have shown that a serine protease present in the lungs is responsible for the activation of F protein and thus for pneumotropism (21, 36, 37).

Recently, we isolated a host range mutant of Sendai virus, F1-R, which underwent multiple cycles of replication in several tissue culture cells, attributed to the proteolytic cleavage of F protein, in the absence of exogenous trypsin (38). Amino acid substitutions near the region of the cleavage site are postulated to confer the increased cleavability by a ubiquitous protease(s) present in several cell types. The mutant was found to be pantropic in mice. Infectious virus was produced and multiple cycles of replication ensued in many organs in addition to the lung (38, 39). These results, taken together with similar observations with virulent and avirulent strains of Newcastle disease virus (23, 24, 40) and avian influenza viruses (2, 3, 13–15), suggest that the structural change in the F glycoprotein of F1-R, with enhanced cleavability, resulted in the broad host range in mice (38, 39). Two questions, however, remain to be answered: (i) whether mouse organs other than the respiratory tract are susceptible to wild-type virus but permissive for F1-R; and (ii) whether the activating protease(s) for wild-type F protein is present only in the respiratory organs, whereas the proteases for F1-R are distributed in the systemic organs.

Sendai virus has been reported to bud exclusively at the apical surface of MDCK cells, a polarized epithelial cell, whereas vesicular stomatitis virus buds at the basolateral region (27). The asymmetrical budding raises the question whether differences in the site of budding in the bronchial

* Corresponding author.

epithelium are a critical factor in the spread of the virus in mice. Thus, progeny virus may spread in the underlying tissues to cause a systemic infection or remain localized in the superficial epithelium. It was therefore of interest to compare the site of budding of wild-type and F1-R viruses.

In this study, we examined the above-stated questions using a mouse organ culture system and immunoelectron microscopy of the bronchial epithelium of mice and of infected MDCK cell monolayers. The results of this study support the view that the proteolytic activation of the F glycoprotein and the domains of budding at the primary target of infection are prime candidates as determinants of organ tropism of Sendai virus.

MATERIALS AND METHODS

Virus. The Z strain of Sendai virus and host range mutants *ts*-f1 and F1-R were propagated and assayed as before (18, 38).

Organ block cultures of mice. Specific-pathogen-free 3-week-old male mice of the ICR strain (CD-1) were obtained from Charles River Japan, Inc. Under anesthesia with ether, mice were bled from the left atrium of the heart with a 1-ml syringe and a 26-gauge needle attached to a three-way plug. Simultaneously, 1 ml of egg-grown virus (4×10^8 PFU/ml) in phosphate-buffered saline containing 0.5% heparin sulfate was injected. The heart stopped beating 3 to 5 min later, and the organs were removed aseptically, minced with a blade, and washed three times with minimal essential medium (MEM). Blocks of tissue (3 mm) were infected again with the same material, and the organ cultures were incubated for 1 h at 33°C. After extensive washing, the tissues were transferred to a 24-well plastic plate and incubated with 0.3 ml of MEM at 33 or 38°C in a 10% CO_2 atmosphere. At various time intervals, medium was collected and organ blocks were homogenized with MEM containing a mixture of protease inhibitors (20 mM phenylmethylsulfonyl fluoride, 20 mM 1-chloro-3-tosylamido-7-amino-L-2-heptanone, 100 kIU of aprotinin per ml). The samples were assayed for infectivity by the differential plaque method in the presence or absence of trypsin in the first overlay agar to determine whether virus was activated (38). Part of the organ block was fixed with 10% Formalin for immunohistological examination as described before (36–38).

Immunoprecipitation, SDS-polyacrylamide gel electrophoresis, and Western immunoblot. Mouse organ cultures frozen at various time intervals and stored at −80°C were solubilized in 2 ml of a lysing buffer (0.5% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 15 mM NaCl, 25 mM Tris hydrochloride, pH 8.0) in the presence of the protease inhibitors. After centrifugation at $1,200 \times g$ for 15 min at 4°C, viral antigens in the supernatant were concentrated by immunoprecipitation. The samples were reacted with 20 μl of anti-Sendai virus mouse serum for 1 h at 4°C, and the immune complexes were absorbed with protein A-conjugated Sepharose CL4B beads (Pharmacia, Uppsala, Sweden) on a rocking device for 60 min at 4°C. The beads were washed three times with 0.1 M NaCl–5 mM EDTA–50 mM Tris hydrochloride, pH 7.4. The absorbed antigens were released from the beads by boiling for 5 min in 200 μl of 1% SDS–1% 2-mercaptoethanol–62.5 mM Tris hydrochloride, pH 6.8. SDS-polyacrylamide gel electrophoresis was done as described previously (36). The polypeptides in the 1.5-mm-thick gel were transferred to Clear Blot Membrane P (ATTO, Tokyo, Japan) by a semidry transfer system at 1.5 mA/cm^2 for 3 h with a transfer buffer (0.1 M Tris, 0.192 M glycine, 20% [vol/vol] methanol, 0.05% SDS). After being blocked with 1% bovine serum albumin, the membrane was treated with anti-Sendai virus rabbit immunoglobulins. For this reaction, antiserum was prepared against egg-grown wild-type virus which was denatured by boiling with 1% SDS–1% 2-mercaptoethanol, because antiserum raised against intact virions did not react quantitatively with monomers of the hemagglutinin-heuraminidase (HN) polypeptide and F_1 subunit on the membrane (data not shown). The membrane was then processed by a sandwich reaction with, successively, biotinylated anti-rabbit immunoglobulin G (heavy plus light chains) goat serum, avidin D, and biotinylated horseradish peroxidase (Vector Laboratories, Inc., Burlingame, Calif.). The viral antigens were visualized by reaction with 4-chloro-1-naphthol and H_2O_2 as the substrates.

Immunoelectron microscopy. Specimens were prepared by the method described previously (6). Briefly, tissues were fixed in periodate-lysine-formaldehyde, and frozen sections were stained by the indirect immunoperoxidase method with rabbit immunogolobulins against Sendai virus glycoprotein fractions (28). The sections were refixed in 1% glutaraldehyde followed by 1% osmium tetroxide postfixation and embedded in Epon-812 araldite. Ultrathin sections were examined in a Hitachi HS9 electron microscope.

Electron microscopy of monolayer cells. Polarized MDCK cell monolayers on polycarbonate membrane filters (tissue culture treated; pore size, 3.0 μm; Costar, Cambridge, Mass.) were infected with wild-type or F1-R virus and incubated in MEM for 13 h at 38°C. Samples were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and examined as described above.

RESULTS

Productive infection of Sendai virus in mouse organ cultures. Since wild-type Sendai virus was recovered after intranasal infection of mice and viral antigens were detected exclusively in the respiratory organs (4, 10, 36, 38), it was particularly of interest to determine whether differences exist among organs of mice in susceptibility to infection by wild-type virus. Intravenous inoculation of mice with more than 10^8 PFU of virus often caused convulsions followed by death immediately after inoculation, presumably because of intravascular agglutination of blood cells in the lung and brain by the virus. Therefore, an organ block culture system was developed so that the virus would infect systemic organs as described in Materials and Methods. In preliminary experiments with wild-type and F1-R viruses, residual infectivity of the inoculum was less than 10^2 PFU/ml in the culture medium and tissue homogenates harvested 3 h after inoculation (data not shown). To assay for virus growth, we harvested the culture medium and organ blocks 24 h after infection and assayed them for infectivity. The infectivity in the medium was about 10 to 50 times lower than that in tissue homogenates. Representative results are presented in Fig. 1. The organs tested produced progeny virus when infected and incubated at 33°C with wild-type, *ts*-f1, and F1-R viruses. Nearly the same yield was noted for the different viruses, although the titer of wild-type virus seemed lower than that of the host range mutants. Replication of *ts*-f1, the temperature-sensitive host range mutant, was negligible, as expected, at 38°C, the mouse body temperature, whereas wild-type and F1-R viruses underwent productive infections, although the yields were lower compared with the infectivity at the lower temperature, 33°C.

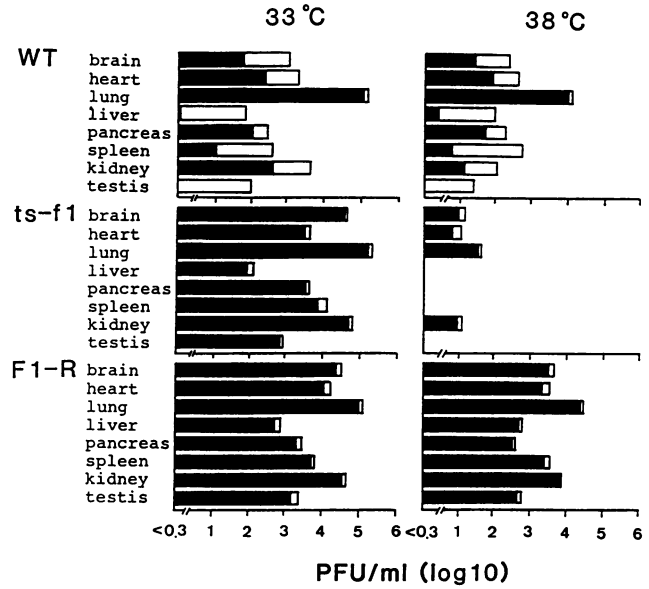

FIG. 1. Production and activation of wild-type (WT), *ts*-f1, and F1-R Sendai viruses in mouse organ cultures. Block cultures of mouse organs infected with the viruses were incubated in MEM for 24 h at 33 or 38°C. The tissue homogenates were assayed for infectivity by the differential plaque method with LLC-MK_2 cells, in the absence or presence of trypsin in the agar overlay, to evaluate whether virus was activated. Black and white bars indicate the amount of activated and nonactivated viruses, respectively. The results show that organs of mice support productive infections of the viruses and that efficient activation of wild-type virus occurs only in the lung, whereas host range mutants are activated in every organ.

Activation of Sendai virus in mouse organ cultures. To estimate the relative number of activated and nonactivated virus particles, we did a differential plaque assay as described in Materials and Methods. The results presented in Fig. 1 show that infectivity of *ts*-f1 and F1-R in the absence of trypsin was about 80% of that in the presence of trypsin. This indicates that progeny virus of the host range mutants was produced predominantly in the activated form in the organs tested. On the other hand, infectivity of wild-type virus was significantly enhanced by trypsin treatment, indicating that substantial amounts of wild-type virus were produced in a nonactive form. The sole exception was in the lung culture, which produced infectious virus as noted previously (36–38). Part of the progeny virus from wild-type virus from the brain, heart, pancreas, and kidney was produced in the activated form but at a significantly lower titer than that with the corresponding virus from organs infected with the host range mutants.

Immunohistology of mouse organ cultures. An immunohistological examination of sections of the organ blocks confirmed that all the organ cultures were certainly infected with wild-type and F1-R viruses (Fig. 2). The distribution of viral antigen-positive cells was similar among cells infected by the three viruses (including *ts*-f1 at 33°C [data not shown]) after 24 h of infection. In general, the epithelial, mesothelial, and vascular endothelial cells were predominantly involved. This is consistent with the finding with F1-R infection in vivo (38, 39). With wild-type virus, however, the results were quite different from infections in vivo, in which viral antigens were detected exclusively in the respiratory epithelium (36). Viral antigen was not detected in any organs after infection with *ts*-f1 at 38°C (data not shown).

Synthesis of viral polypeptides and proteolytic cleavage of F glycoprotein in organ cultures. To verify whether activation of Sendai viruses in different organs resulted in the proteolytic cleavage of the F glycoprotein, we did Western blot analyses. Viral polypeptides were initially detected in the organ cultures 3 h after infection (data not shown). The results presented in Fig. 3 were obtained from cultures incubated for 24 h at 38°C. The viral polypeptides were detected in the organ cultures infected with wild-type and F1-R viruses. Cleavage of the F glycoprotein of wild-type virus occurred in the lung and, to a lesser degree, in the brain, heart, pancreas, and kidney. Proteolytic cleavage of the F protein did not occur in the other organs. On the other hand, the F protein of F1-R was cleaved in the organs as indicated by F_1 and F_2; some uncleaved F protein was detected. The differences in the migration of F protein bands F_0 and F_2 are consistent with the finding that the F_2 subunit of F1-R is lacking in a carbohydrate side chain (38, 39). Confirmation of the phenotype of the M protein of F1-R, with a faster-migrating band, is also evident (38). Identical protein profiles of *ts*-f1 and F1-R were obtained in organ cultures incubated at 33°C (data not shown).

Site of budding by Sendai viruses in epithelial cells of mouse bronchus. The site of budding in infected bronchial epithelium, the primary target of natural infections, was compared between wild-type and F1-R viruses by immunoelectron microscopy. Mouse lungs were collected 24 h after intranasal infections. In the bronchial epithelial cells infected with wild-type virus, viral glycoprotein antigens were detected predominantly at the apical surface of the plasma membrane, suggesting that budding occurred at this domain (Fig. 4A). However, in F1-R-infected cells, viral antigens were observed at both the apical and basolateral domains. Viral particles of F1-R were also seen beneath the infected epithelial cells and surrounding the lining of the basement membrane (Fig. 4B, C, and D). The different localization of viral antigens between the viruses is consistent with previous observations of immunohistology (36, 38).

Site of budding of Sendai viruses in MDCK cell monolayers. To confirm the bidirectional budding observed in the bronchial epithelium infected with F1-R, we infected MDCK cell monolayers and examined them by electron microscopy. The electron micrographs presented in Fig. 5 illustrate budding of F1-R at both the apical and basolateral domains of the plasma membrane (Fig. 5A, B, and C), whereas wild-type virus budded exclusively at the apical domain (Fig. 5D). The latter finding was identical to the previous report (27).

DISCUSSION

Our data show that not only the pantropic variant, F1-R, but also pneumotropic wild-type Sendai virus was able to infect and undergo productive infections in various organs of mice, if the organs were infected with the viruses. Viral polypeptides were synthesized and progeny viruses were assembled, in the active or nonactive form, under conditions corresponding to one cycle of replication (Fig. 1 and 3). Thus, organs in addition to the lung are potentially susceptible to wild-type virus. These findings may rule out the possible mechanism of pneumotropism in which organs of mice other than the respiratory tract are not susceptible to wild-type virus. Although specific cell types which produced progeny virus have not been identified in the individual organs, the distribution of viral antigen-positive cells was similar in cells infected with wild-type, F1-R, and *ts*-f1

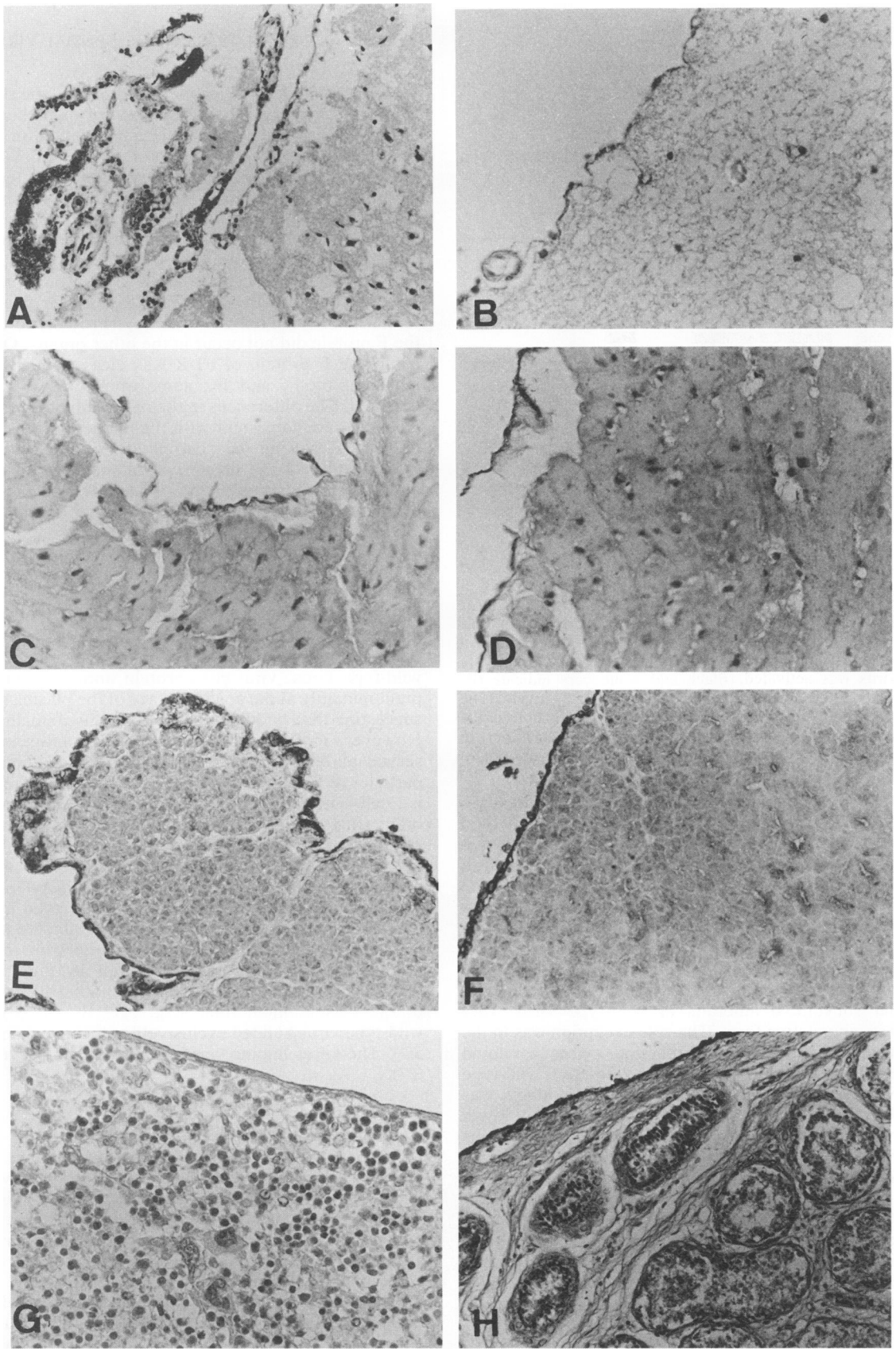

FIG. 2. Immunohistology of mouse organ cultures infected with Sendai viruses. Block cultures of mouse organs were infected with wild-type or F1-R virus and incubated in MEM for 24 h at 38°C. The tissue blocks were fixed in 10% Formalin, and thin sections were stained for Sendai virus antigens by the immunoperoxidase method with anti-Sendai virus rabbit serum (36). The nuclei were counterstained with methyl green. (A and B) Brain; (C and D) heart; (E and F) pancreas; (G) spleen; (H) testis. (A, C, E, G, and H) Wild-type virus-infected organs; (B, D, and F) F1-R-infected organs. Magnification, ×184 (A, B, E, F, and H), ×368 (C, D, and G).

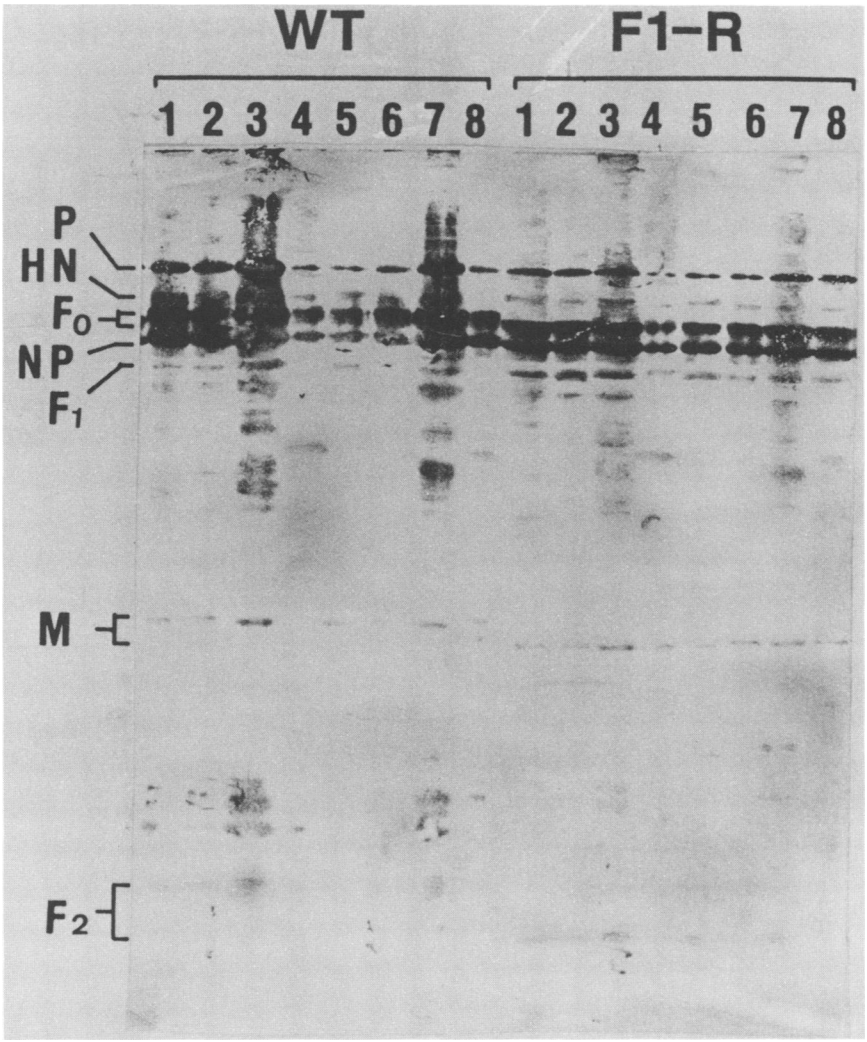

FIG. 3. Western blot analysis of mouse organ cultures infected with wild-type (WT) and F1-R Sendai viruses. Block cultures of mouse organs infected with the viruses were incubated for 24 h in MEM at 38°C. Tissues were homogenized, and viral antigens were condensed by the immunoprecipitation method. Samples (360 μg for each lane) were analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blotting. Viral polypeptides on the membrane were reacted with the anti-Sendai virus rabbit serum and visualized by the peroxidase reaction. The F protein of wild-type virus from the lung shows proteolytic cleavage, and that from the brain, heart, pancreas, and kidney is cleaved slightly. All lanes of F1-R show cleavage of F protein. The phenotype of M protein (abnormal migration) for F1-R has been confirmed. Lanes 1 to 8, Brain, heart, lung, liver, pancreas, spleen, kidney, and testis, respectively.

viruses (Fig. 2) and infections in vivo with F1-R (38). Since wild-type virions were produced in the organs tested, mutations found in the NP, P, M, and L genes of the host range mutants (Y. Middleton, M. Tashiro, T. Thai, J. Oh, J. Seymour, E. Pritzer, H.-D. Klenk, R. Rott, and J. T. Seto, Virology, in press) may not be of significance in virus production in mice. Additionally, the HN gene of F1-R and *ts*-f1 was devoid of any mutations (Middleton et al., in press). Thus, the host range mutants are able to attach to the receptors for wild-type virus, which are widely distributed in the organs of mice (11). Additionally, any association between increased neurovirulence in mice and low neuraminidase activity, as reported for bovine parainfluenza virus type 3, mumps virus, and the WSN strain of influenza virus (19, 31, 35), may not apply to Sendai virus.

Since *ts*-f1-infected organs at permissive temperature supported multiple cycles of replication similar to those of F1-R, *ts*-f1 could also cause a generalized infection in mice if the body temperature was as low as 33°C. Identical mutations occurred in the F and M genes of the host range mutants (38, 39; Middleton et al., in press).

Since progeny virus of F1-R was produced in the activated form in the organ cultures (Fig. 1), the mutant must undergo multiple cycles of replication in the same organs in vivo (38, 39). It should be emphasized that, in contrast to the mutant, wild-type virus remained nonactive in most organs, particularly in the liver, spleen, and testis. Hence, these organs are unlikely to support multiple cycles of replication of wild-type virus in vivo even if they are infected. On the other hand, virus was readily activated in the lung (Fig. 1 and 3), supporting multiple steps of replication of the virus as reported previously (36–38). These findings indicate that the wild-type F glycoprotein was partially activated by a ubiquitous protease(s), whereas the F protein of F1-R was completely activated. The activating enzyme for wild-type F protein must be localized in the respiratory tract. Therefore,

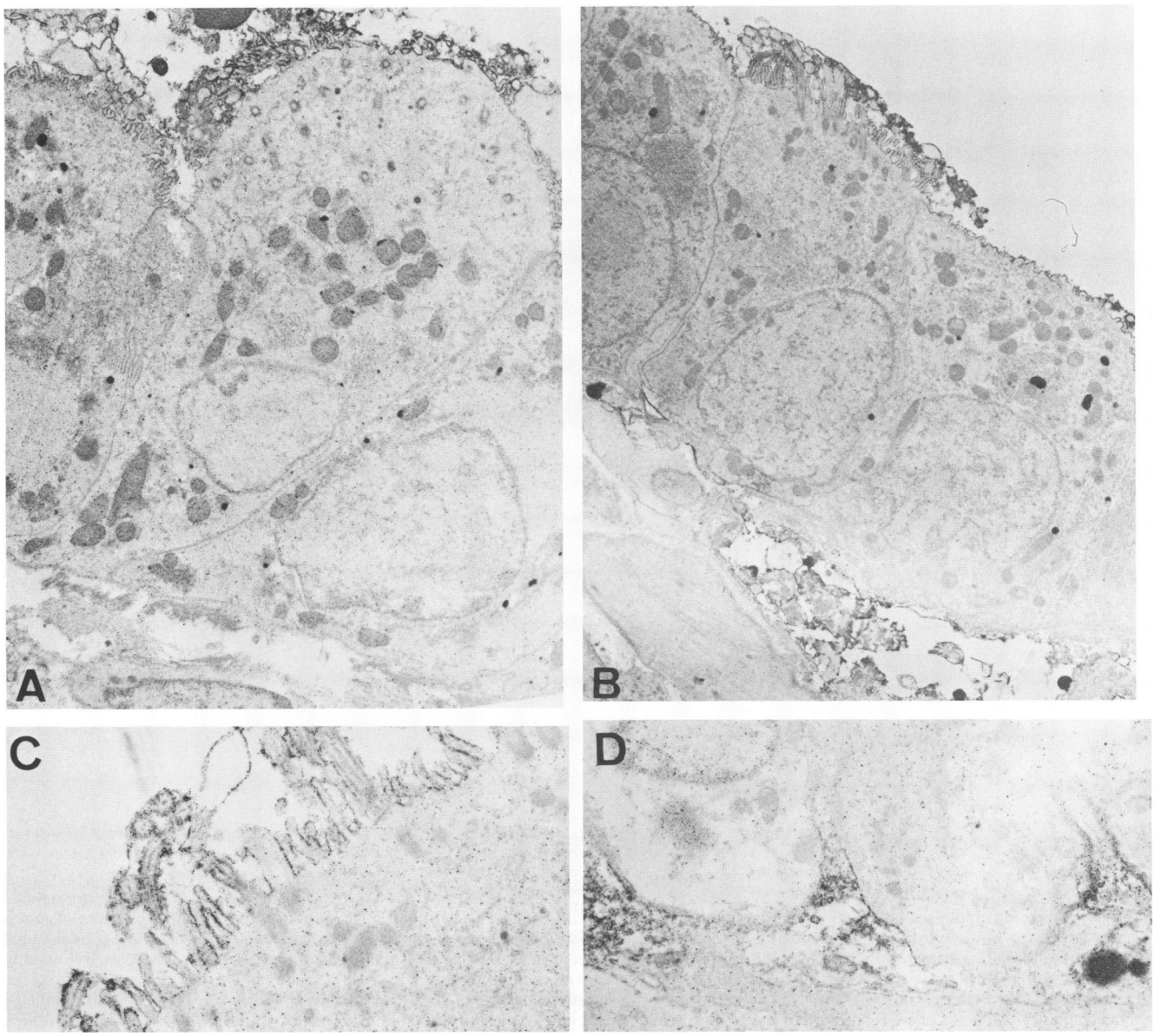

FIG. 4. Immunoelectron microscopy of mouse bronchial epithelium infected with wild-type and F1-R viruses. Mice were infected intranasally with the viruses for 24 h, and the lungs were processed for immunoelectron microscopy with antibody against viral glycoproteins. (A) Wild-type viral antigens are evident at the apical surface of the bronchial epithelial cells. (B, C, and D) F1-R viral antigens are evident at the apical (C) and basal (D) regions; aggregations of viral particles are found beneath the epithelium. Magnification, ×4,500 (A and B), ×9,600 (C and D).

our data strongly suggest that the different distribution of activating proteases for F glycoprotein in organs of mice is the principal determinant for pneumotropism of wild-type Sendai virus and for pantropism of the protease activation mutant, F1-R. Information about the specific cell types that activate the F proteins of wild-type and F1-R viruses is not available at present. Likewise, the distribution and the precise nature of the activating protease(s) for Sendai viruses in organs of mice remain to be determined. Serum plasmin might be an activator for F1-R because it augments infectivity of the mutant but not of wild-type virus (38, 39).

Part of the progeny of wild-type virus was produced in the activated form in the brain, heart, pancreas, and kidneys, even though the titers were significantly lower than those of F1-R (Fig. 1 and 3). These organs, therefore, might be able to support multiple cycles of replication of wild-type virus to some extent if they were infected. In newborn mice, wild-type virus has been reported to infect the brain after intracranial inoculation (17, 20, 32), and intranasal infection has led to spread of virus to the brain via the olfactory nerve (16). However, wild-type virus was recovered only from the respiratory tract in weaning or adult mice after intranasal infection. These findings suggest that the differential capacity of organs in mice to activate the F glycoprotein, although essential, may be viewed as requiring an additional factor(s) for organ tropism.

The observation of the polarized budding by wild-type virus and bidirectional budding by F1-R in the bronchial

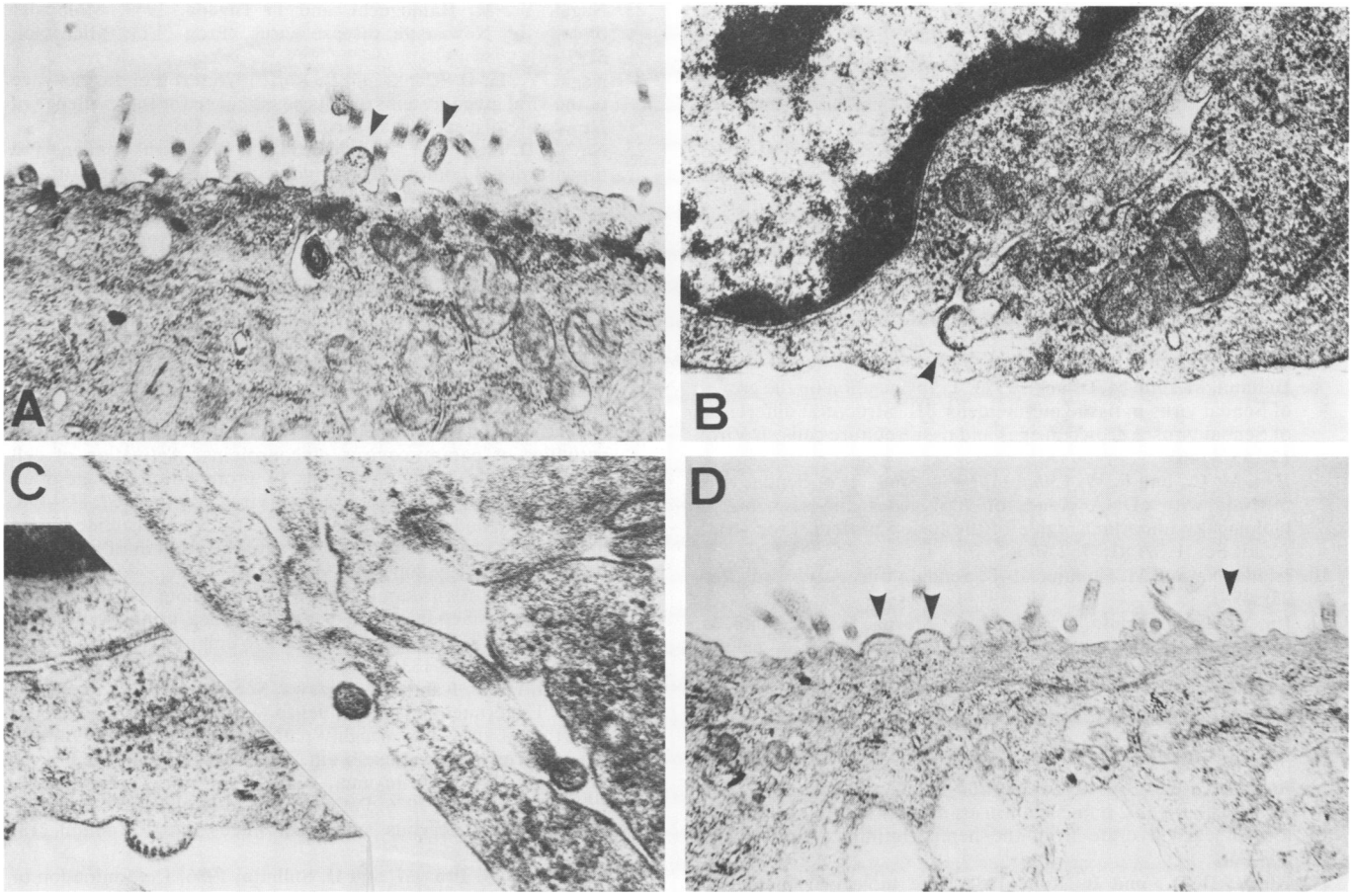

FIG. 5. Electron micrographs of MDCK cells infected with Sendai viruses. Polarized MDCK cell monolayers on polycarbonate filter membranes were infected with wild-type and F1-R viruses and incubated for 13 h in MEM at 38°C. (A) Apical plasma membrane with budding F1-R virus (arrowheads). Magnification, ×32,000. (B) Basal domain of the plasma membrane with F1-R budding into the lateral space (arrowhead); ×46,000. (C) Basal plasma membranes with budding F1-R virions; ×51,000. Inset, ×82,000. (D) Apical plasma membrane with budding wild-type virus (arrowheads); ×32,000.

epithelium of mice (Fig. 4) suggests that the mode of budding at the primary target of infection is an additional determinant for organ tropism. Although the question remains to be answered whether viral particles released from the basal domain are infectious, it is a likely possibility since infectious progeny of F1-R were recovered from both apical and basolateral regions of MDCK cell monolayers (M. Tashiro, M. Yamakawa, K. Tobita, J. T. Seto, H.-D. Klenk, and R. Rott, submitted for publication). Pneumotropism of wild-type virus may be determined by its budding at the apical surface of bronchial epithelium of mice. On the other hand, infection by F1-R that also buds from the basolateral region of the cell domain may explain how virus readily invades the bloodstream through subepithelial tissues and lymphatics to cause viremia. The target cells in the distant organs could then become infected and support multiple cycles of replication of the mutant.

The bidirectional budding of F1-R revealed in the bronchial epithelium of mice and in monolayers of MDCK cells, previously not observed for viruses, is of importance in the identification of signals responsible for the transport mechanisms of viral transmembrane proteins (25). Because identical mutations in the M gene of the host range mutants have led to a change in the structure of the M protein (Middleton et al., in press) and because of the importance of M protein in the assembly and maturation of the virus, the possible interaction of M protein with F protein must be considered as a prime candidate for the altered budding and thus as an additional determinant for pantropism of F1-R. Studies are in progress to investigate this possibility.

ACKNOWLEDGMENTS

This work was supported by grants-in-aid for scientific research from the Minister of Education, Science and Culture, Japan; by Public Health Service grants RR 08101-18 (MRBS program) and AI-24096 from the National Institutes of Health; by the Deutsche Forschungsgemeinschaft; and by the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

LITERATURE CITED

1. **Blumberg, B. M., C. Giorgi, K. Rose, and D. Kolakofsky.** 1985. Sequence determination of the Sendai virus fusion protein gene. J. Gen. Virol. **66**:317–331.
2. **Bosch, F. X., W. Garten, H.-D. Klenk, and R. Rott.** 1981. Proteolytic cleavage of influenza virus hemagglutinin. Primary structure of the connecting peptide between HA_1 and HA_2 determines proteolytic cleavability and pathogenicity of avian influenza viruses. Virology **113**:725–735.
3. **Bosch, F. X., M. Orlich, H.-D. Klenk, and R. Rott.** 1979. The

structure of the hemagglutinin, a determinant for the pathogenicity of influenza viruses. Virology **95**:197–207.

4. **Brownstein, D. G.** 1986. Sendai virus, p. 37–61. *In* P. N. Bhatt, R. O. Jacoby, and H. C. Morse III (ed.), Viral and mycoplasmal infections of laboratory rodents. Effects on biochemical research. Academic Press, Inc., Orlando, Fla.
5. **Choppin, P. W., and A. Scheid.** 1980. The role of viral glycoproteins in adsorption, penetration and pathogenicity of viruses. Rev. Infect. Dis. **2**:40–61.
6. **Farr, A. F., and P. K. Nakane.** 1981. Immunohistochemistry with enzyme labeled antibodies: a brief review. J. Immunol. **47**:129–144.
7. **Homma, M.** 1971. Trypsin action on the growth of Sendai virus in tissue culture cells. I. Restoration of the infectivity for L cells by direct action of trypsin on L cell-borne Sendai virus. J. Virol. **8**:619–629.
8. **Homma, M., and M. Ohuchi.** 1973. Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural differences of Sendai viruses grown in eggs and tissue culture cells. J. Virol. **12**:1457–1465.
9. **Hsu, M. C., and P. W. Choppin.** 1984. Analysis of Sendai virus mRNAs with cDNA clones of viral genes and sequence of biologically important region of the fusion protein. Proc. Natl. Acad. Sci. USA **81**:7732–7736.
10. **Ishida, N., and M. Homma.** 1978. Sendai virus. Adv. Virus Res. **23**:349–383.
11. **Ito, Y., F. Yamamoto, M. Takano, K. Maeno, K. Shimokata, M. Iinuma, K. Hara, and S. Iijima.** 1983. Detection of cellular receptors for Sendai virus in mouse tissue section. Arch. Virol. **75**:103–113.
12. **Itoh, M., H. Shibuta, and M. Homma.** 1987. Single amino acid substitution of Sendai virus at the cleavage site of the fusion protein confers trypsin resistance. J. Gen. Virol. **68**:2939–2944.
13. **Kawaoka, Y., C. W. Naeve, and R. G. Webster.** 1984. Is virulence of H5N2 influenza viruses in chickens associated with loss of carbohydrate from the hemagglutinin? Virology **139**: 303–316.
14. **Klenk, H.-D., and R. Rott.** 1988. The molecular biology of influenza virus pathogenicity. Adv. Virus Res. **34**:247–281.
15. **Klenk, H.-D., M. Tashiro, W. Garten, and R. Rott.** 1988. Viral glycoproteins as determinants of pathogenicity, p. 25–38. *In* R. Rott and W. Goebel (ed.), Molecular bases of viral and microbial pathogenesis. Springer-Verlag KG, Berlin.
16. **Kristensson, K., J. Leestma, B. Lundh, and E. Norrby.** 1984. Sendai virus infection in the mouse brain: virus spread and long-term effects. Acta Neuropathol. **63**:89–95.
17. **Kristensson, K., C. Örvell, J. Leestma, and E. Norrby.** 1983. Sendai virus infection in the brain of mice: distribution of viral antigens studied with monoclonal antibodies. J. Infect. Dis. **147**:297–301.
18. **Lawton, P., Z. Karimi, L. Mancinelli, and J. T. Seto.** 1986. Persistent infections with Sendai virus and Newcastle disease viruses. Arch. Virol. **89**:225–233.
19. **Merz, D. C., and J. S. Wolinsky.** 1981. Biochemical features of mumps virus neuraminidase and their relationship to pathogenicity. Virology **114**:218–227.
20. **Mims, C. A., and F. A. Murphy.** 1973. Parainfluenza virus Sendai infection in macrophages, ependyma, choroid plexus, vascular endothelium and respiratory tract of mice. Am. J. Pathol. **70**:315–328.
21. **Mochizuki, Y., M. Tashiro, and M. Homma.** 1988. Pneumopathogenicity in mice of a Sendai virus mutant, TSrev-58, is accompanied by in vitro activation with trypsin. J. Virol. **62**:3040–3042.
22. **Muramatsu, M., and M. Homma.** 1980. Trypsin action on the growth of Sendai virus in tissue culture cells. V. An activating enzyme for Sendai virus in the chorioallantoic fluid of the embryonated chicken eggs. Microbiol. Immunol. **24**:113–122.
23. **Nagai, Y., M. Hamaguchi, and T. Toyoda.** 1989. Molecular biology of Newcastle disease virus. Prog. Vet. Microbiol. **5**:16–64.
24. **Nagai, Y., H.-D. Klenk, and R. Rott.** 1976. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology **72**:494–508.
25. **Nayak, D. P., and M. A. Jabbar.** 1989. Structural domains and organizational conformation involved in the sorting and transport of influenza virus transmembrane proteins. Annu. Rev. Microbiol. **43**:461–501.
26. **Portner, A., R. A. Scroggs, and C. W. Naeve.** 1987. The fusion glycoprotein of Sendai virus: sequence analysis of an epitope involved in fusion and virus neutralization. Virology **157**:556–559.
27. **Rodriguez-Boulan, E., and D. D. Sabatini.** 1978. Asymmetric budding of viruses in epithelial monolayers: a model for the study of epithelial cell polarity. Proc. Natl. Acad. Sci. USA **75**:5071–5075.
28. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475–490.
29. **Scheid, A., and P. W. Choppin.** 1977. Two disulfide-linked polypeptide chains constitute the active F protein of paramyxoviruses. Virology **80**:54–66.
30. **Shibuta, H., M. Akami, and M. Matsumoto.** 1971. Plaque formation by Sendai virus of parainfluenza group, type 1 on monkey, calf kidney and chick embryo cell monolayers. Jpn. J. Microbiol. **15**:175–183.
31. **Shibuta, H., T. Kanda, A. Nazawa, S. Sato, and T. Kumanishi.** 1985. Experimental parainfluenza virus infection in mice: growth and spread of a highly pathogenic variant of parainfluenza 3 virus in the mouse brain. Arch. Virol. **83**:43–52.
32. **Shimokata, K., Y. Nishiyama, Y. Ito, Y. Kimura, I. Nagata, M. Iida, and I. Sobue.** 1976. Pathogenesis of Sendai virus infection in the central nervous system of mice. Infect. Immun. **13**: 1497–1502.
33. **Shioda, T., K. Iwasaki, and H. Shibuta.** 1986. Determination of the complete nucleotide sequence of the Sendai virus genome RNA and the predicted amino acid sequences of the F, HN and L proteins. Nucleic Acids Res. **14**:1545–1563.
34. **Silver, S. M., A. Scheid, and P. W. Choppin.** 1978. Loss on serial passage of rhesus monkey kidney cells of proteolytic activity required for Sendai virus activation. Infect. Immun. **20**:235–241.
35. **Sugiura, A.** 1975. Influenza virus genetics, p. 171–213. *In* E. D. Kilbourne (ed.), Influenza viruses and influenza. Academic Press, Inc., Orlando, Fla.
36. **Tashiro, M., and M. Homma.** 1983. Pneumotropism of Sendai virus in relation to protease-mediated activation in mouse lungs. Infect. Immun. **39**:879–888.
37. **Tashiro, M., and M. Homma.** 1983. Evidence of proteolytic activation of Sendai virus in mouse lung. Arch. Virol. **77**: 127–137.
38. **Tashiro, M., E. Pritzer, M. A. Khoshnan, M. Yamakawa, K. Kuroda, H.-D. Klenk, R. Rott, and J. T. Seto.** 1988. Characterization of a pantropic variant of Sendai virus derived from a host-range mutant. Virology **165**:577–583.
39. **Tashiro, M., E. Pritzer, M. A. Khoshnan, M. Yamakawa, K. Kuroda, H.-D. Klenk, R. Rott, and J. T. Seto.** 1989. Pantropic variant of Sendai virus: a mutational change in the proteolytic cleavability of the fusion glycoprotein alters organtropism in mice, p. 341–347. *In* D. Kolakofsky and B. Mahy (ed.), Genetics and pathogenicity of negative strand viruses. Elsevier Biomedical Press, Amsterdam.
40. **Toyoda, T., T. Sakaguchi, K. Imai, N. M. Inocencio, B. Gotoh, M. Hamaguchi, and Y. Nagai.** 1987. Structural comparison of the cleavage-activation site of the fusion glycoprotein between virulent and avirulent strains of Newcastle disease virus. Virology **158**:242–247.