PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 142–149 (1987)

Measles Virus Persistence in a Hamster Brain Cell Line: Cyclical Fluctuation of Viral Expression during Serial Passages (42594)

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Abstract. Measles virus persistence in a hamster brain cell line was examined for the appearance of viral antigens and for the synthesis of infectious virus, viral RNA, and virus-specific proteins during 19 serial passages. Cyclical fluctuation was detected at all levels of measles virus replication. After reaching the maximal activity at the passages 9 to 11 viral synthesis diminished rapidly, being lowest after 2 to 3 subsequent passages. No release of infectious virus was detected at the passages 14 and 15 and only 20% of the cells contained measles virus antigens when tested by immunofluorescence. After the nonproductive phase, the cells released virus again and the number of antigen-positive cells increased. The alternation in the amount of the measles virus-specific proteins and viral RNA correlated directly with the parameters mentioned above. No apparent defects in the synthesis of individual viral proteins were observed.

Measles virus (MV) causes a well-known acute illness which relatively often is complicated with symptoms in the central nervous system (CNS). In cell cultures inoculated with the virus a lytic infection with giant cell (syncytium) formation and cell death is observed. In addition, measles virus is known to generate persistent infections both *in vivo* and *in vitro*. The fatal human neurological disease, subacute sclerosing panencephalitis (SSPE), has been shown to be associated with chronic measles virus infection of the CNS (1).

In vitro, different types of persistently infected cell lines have been established. Some of these cell lines produce infectious virus while others do not, even though the cells contain high amounts of intracellular viral antigens. A balance must be maintained between virus replication and cell survival, as persistently infected cell lines may be cultivated for years with continuous expression of viral antigens and production of infectious virus. The mechanism behind this virus–host cell interaction is, however, poorly understood. Several different viral specific factors (temperature-sensitive mutants, defective interfering particles, interferon, and alterations in the viral structural proteins) and host-specific events have been suggested to play roles in the induction and maintenance of the persistent state.

The amount and antigenicity of the MV matrix (M) protein has been reported to be altered in brain tissues of SSPE patients (2–5) and in a hamster model during subacute measles infection (6). The lack of hemadsorption activity or of the hemagglutinin glycoprotein H have also been described in SSPE brains and in cell lines persistently infected with MV SSPE strains (7, 8). Recently, Norrby *et al.* (9) reported the appearance of both the matrix protein and the hemagglutinin glycoprotein in brain material from SSPE patients.

In order to understand this complex virus–host cell interaction, we have studied MV persistence in a cloned hamster brain cell line during 19 serial passages. Although the immune system adds further complexity *in vivo* brain cells as host cells serve as a good model to study *in vitro* persistence. In this report we describe a cyclical fluctuation of the production of infectious MV and of the expression of viral RNA and proteins in the cell line during the observation period. Our results support the suggestion that in the hamster brain cell line, virus replication is complete but other factor(s), possibly including those host cell mediated, are involved in the regulation of the balance of virus–host interaction.

Materials and Methods. *Establishment and cloning of the hamster brain cell line persis-*

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0037-9727/87 $1.50

tently infected with measles virus. Establishment of the hamster brain cell line persistently infected with a wild-type measles virus has been described earlier (10). In brief, brain tissue from newborn hamsters was cut into small pieces, explanted onto petri dishes, and maintained in modified Eagle's medium (MEM) supplemented with 20% fetal calf serum (FCS) and antibiotics. When the dividing cells covered the bottom of the dish, human papova virus BK was added as a transforming agent. The transformed hamster brain cell cultures were then infected with a wild-type measles virus at a multiplicity of infection of 0.01 PFU/cell. The virus-infected hamster brain cell line and a similarly established noninfected control brain cell line were cloned to a one-cell level in agarose. The cloned measles virus-infected (MV-308) cell line was trypsinized twice a week and maintained in MEM supplemented with 5% FCS and antibiotics. For this study the number of cells in each passage was counted and the protein concentration was determined. The collected specimens were stored at −70°C until analyzed.

Infectivity titration and immunofluorescence. The amount of infectious extracellular virus was determined by incubation of serial 10-fold dilutions of culture supernatants on confluent monolayers of Vero cells. Each dilution was assayed in duplicate. The results were obtained after an incubation period of 10 days and the amount of virus is expressed as endpoint titers.

For immunofluorescence, the cells were detached mechanically, centrifuged onto a glass slide in a cytocentrifuge, and fixed with cold acetone. The slides were stored at −70°C until stained with rabbit anti-measles serum (1:500 dilution) for 30 min at 37°C followed by sheep anti-rabbit immunoglobulin conjugated with fluorescein isothiocyanate (FITC, Wellcome, Beckenham, Kent, UK). The preparations were examined with a Leitz Dialux 22 microscope.

Electron microscopy. For intracellular immunoperoxidase staining, the cells were grown in monolayers and fixed in 3% paraformaldehyde, 0.05% glutaraldehyde, and 0.05% saponin in 0.1 *M* phosphate-buffered saline (PBS) for 30 min at 4°C. After prefixation, the cells were washed with Dulbecco's PBS containing 0.2% bovine serum albumin (BSA), 0.05% saponin, and 0.05 *M* lysine–HCl. One part of the cell preparation was incubated with rabbit anti-measles nucleocapsid serum (1:50 dilution) and the other part with normal rabbit serum (1:50 dilution) at 4°C. After 1 hr, the cells were washed and incubated with peroxidase-conjugated staphylococcal protein A as described previously (11) with the exception that 2.5% glutaraldehyde in S-collidine–HCl buffer, pH 7.4, was used for postfixation. After labeling, the cells were embedded in Ladd's resin. Thin sections were prepared, stained with 12.5% uranyl acetate and 0.25% lead citrate, and examined in a Philips EM 410 electron microscope.

Analysis of measles virus-specific proteins. The amount of measles virus-specific polypeptides in MV-308 cells was analyzed by dot-blotting as previously described (12) with the exception that 25 μg of protein/specimen was spotted in 0.1 *M* Tris–HCl buffer, pH 6.8, containing 2% sodium dodecyl sulphate (SDS) on a nitrocellulose filter (0.45 μ*M*, BA85, Schleicher & Schuell Co., Dassel, Federal Republic of Germany). The relative amount of each antigen was detected by incubating the filters overnight at 4°C with a high-titer human anti-measles serum, diluted 1:500, followed by ^{125}I-labeled anti-human serum (2×10^4 cpm/ml). The bound radioactivity was detected both by autoradiography and gamma counting.

The synthesis of measles virus-specific proteins was studied by radiolabeling the cell cultures metabolically with [^{35}S]methionine as described earlier (10). The cells were first cultured with methionine-free MEM for 30 min, followed by methionine-free medium containing 50 μCi/ml [^{35}S]methionine (sp act 1405 Ci/m*M*, Amersham, England) for a further 30 min. At the end of the labeling period, the cells were washed twice with 0.1 *M* Tris–HCl, pH 6.8, and lysed in the same buffer containing 2% SDS. DNA was shared mechanically and the acid-insoluble radioactivity was determined.

The MV-specific proteins were studied by the Western blotting technique as described previously (12). The protein/specimen (25 μg) was analyzed by 10% SDS–polyacrylamide gel electrophoresis, after which the polypeptides were transferred onto a nitrocellulose filter (0.22 μm, BA85, Schleicher & Schuell Co.)

and incubated with rabbit anti-measles virus serum (1:500 dilution). The bound antibody was visualized with horseradish peroxidase-conjugated anti-rabbit serum and with the precipitating color substrate (10 mg 3-amino-ethylcarbazole in 2.5 ml *N,N*-dimethylformamide diluted with 47.5 ml of 50 m*M* sodium acetate buffer, pH 5.0, and 50 μl H_2O_2 added just before use).

Determination of viral RNA. Cells (10^6) were treated with proteinase K (E Merck AG, Darmstadt, Federal Republic of Germany) at a concentration of 0.1 mg/ml in PBS. After incubation for 1 hr at 37°C the specimens were adjusted to contain 0.9 *M* NaCl and 0.09 *M* sodium citrate (6× SSC) and spotted on a nitrocellulose filter (0.45 μm, BA85, Schleicher & Schuell Co.). The filters were baked for 2 hr at 80°C and prehybridized for 2 hr at 42°C in 50% formamide, 3× SSC, 50 m*M* Hepes, pH 7.4, 0.1% SDS, 250 μg of yeast RNA/ml, and 100 μg of denatured herring sperm DNA/ml. The probe was prepared by a random-primed reaction (13) using measles viral RNA as a template and [^{32}P]dCTP (sp act 3000 Ci/mM, Amersham, England) as the isotope. The probe, with a specific activity of approximately 1×10^7 cpm/ml, was denatured, added directly to the prehybridization solution, and incubated overnight at 42°C. The filter was washed in 15 m*M* NaCl, 1.5 m*M* sodium citrate containing 0.1% SDS, for 1 hr at 65°C, and the radioactivity was detected either by autoradiography overnight or by scintillation counting.

Results.*General properties of the MV-308 hamster brain cell line.* The MV-308 cell line is a constant carrier cell line of measles virus with a good growth capacity. Our data concern passages 1 to 19, but the cell line has now been

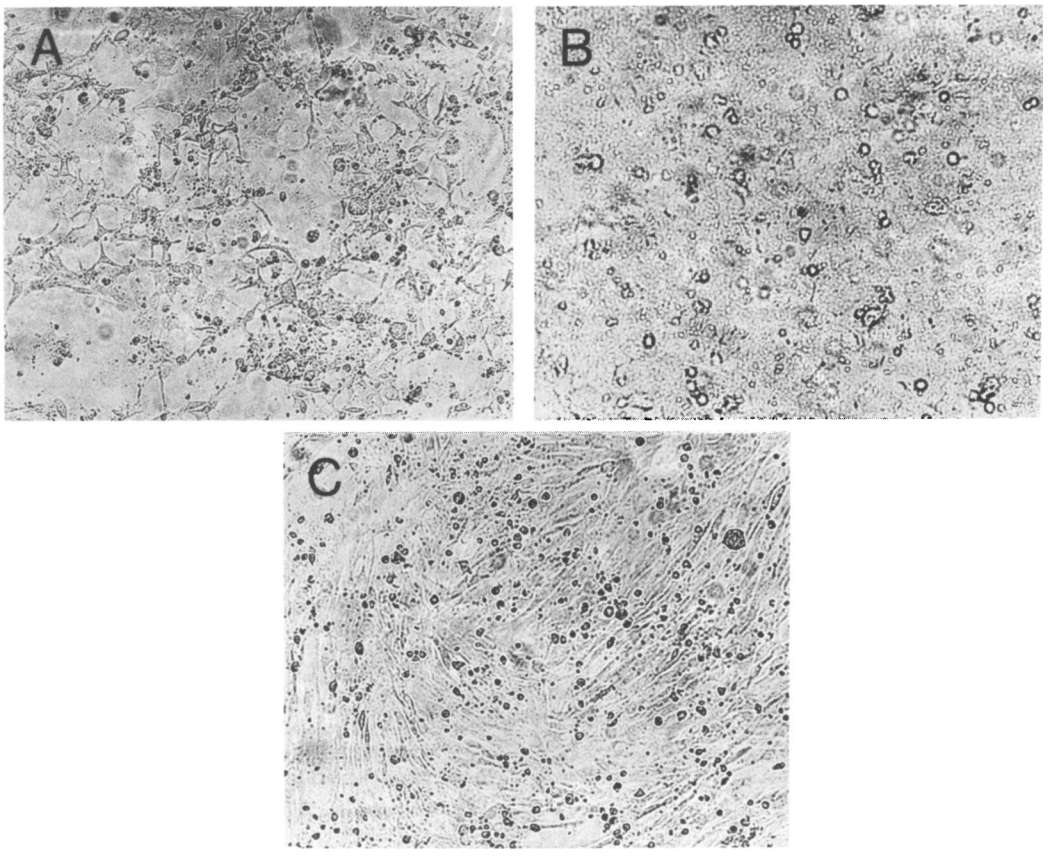

FIG. 1. Morphology of the MV-308 hamster brain cells at passages 7 (A) and 19 (B). (C) is the control cell line at passage 7.

passaged for more than 100 times. The cytopathology was typical in all the passages and clear syncytia were seen with certain intervals (Fig. 1). However, the majority of cells survived and could be passaged twice a week. The control cell line 102 was trypsinized once a week. The effect of incubation temperature on the expression of measles virus in hamster

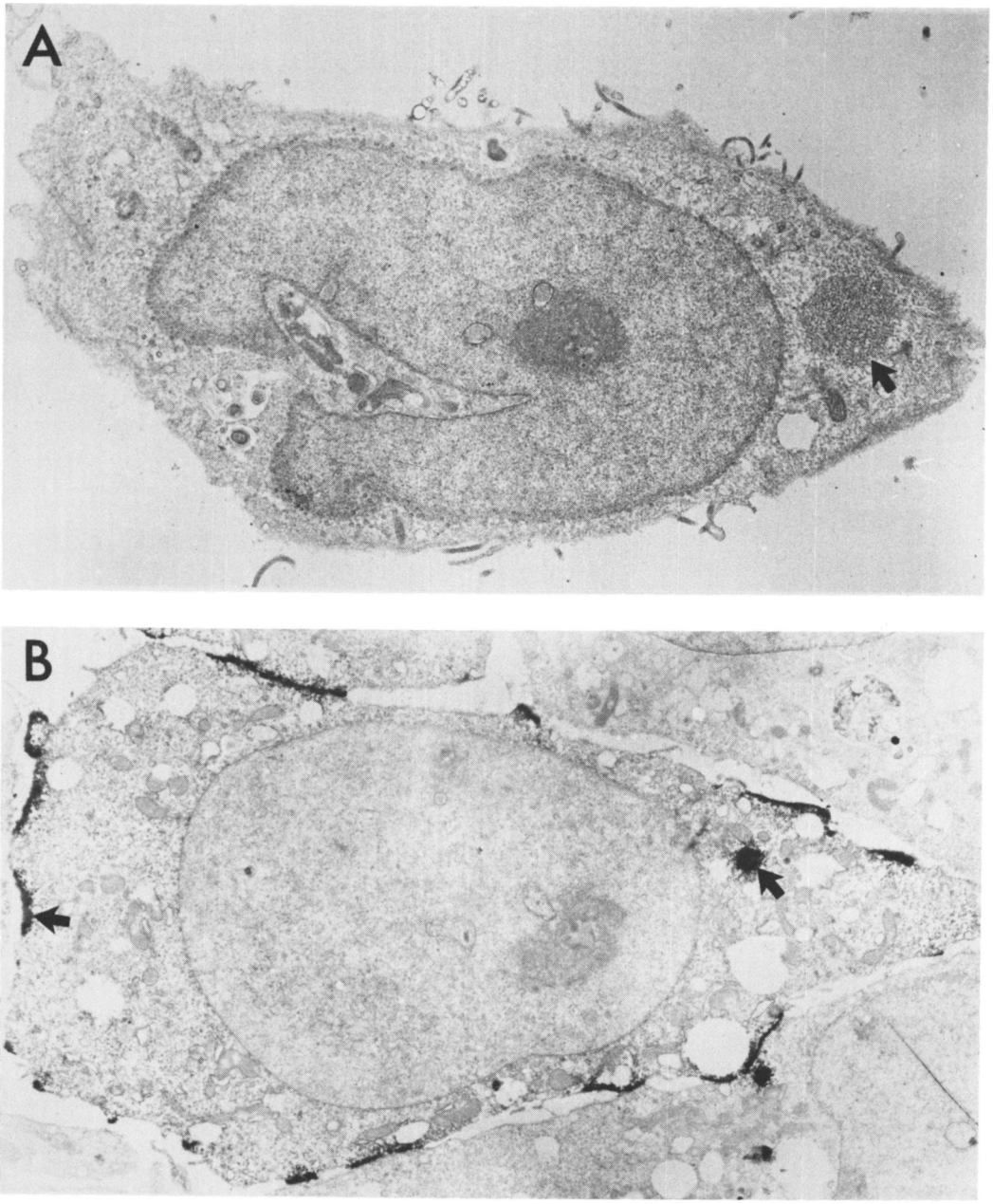

FIG. 2. Ultrastructure of hamster brain cells persistently infected with measles virus. (A) stained with normal rabbit serum and HRPO-conjugated staphylococcal protein A and (B) with rabbit antinucleocapsid serum and HRPO-conjugated staphylococcal protein A. Nucleocapsid structures in the cytoplasm and on plasma membrane are indicated with arrows.

brain cells has been analyzed previously (10) with no detectable effects between 32 and 37°C. After cloning, the cells were grown at 37°C. The data presented here concern the MV-308 cell line, but comparable results have also been obtained with another cloned hamster brain cell line, MV-23. Only the MV cyclic fluctuation in MV-23 cells is lower.

In electron microscopy, nucleocapsid structures were observed in the cytoplasm of the MV-308 cells (Fig. 2A) and by immunological staining nucleocapsid clusters were also seen in association with the plasma membrane (Fig. 2B). No clear nuclear accumulation of nucleocapsids was observed by electron microscopic study.

The MV-308 cell line produced infectious virus virtually continuously. However, the amount of extracellular infectious virus varied cyclically during the observation period, reaching a maximum titer of 5.5×10^5 TCID/ml at passages 9 to 11 and then switching to a nonproductive phase by passages 14 to 15 (Fig. 3A). Similar cyclical fluctuation was also observed in subsequent passages (data not shown). The recovered virus had similar growth properties and formed similar cytopathogenic changes in Vero cells when compared with the original wild-type virus used to establish the persistent cell line.

We have earlier reported (10) that the hamster brain cell line M-HB/MVB, from which the MV-308 cell line has been derived, secreted a small amount of interferon into the culture supernatant. When the culture supernatants from serial MV-308 passages were tested in an interferon assay (14), the results were negative.

Immunofluorescence. In order to study whether the fluctuation in the amount of extracellular infectious virus were the result of alternations in virus release or in the proportional amount of measles virus infected cells in serial passages, the number of fluorescence-positive cells was determined using measles virus antiserum. The number of positive cells fluctuated in parallel with the amount of extracellular virus produced (Fig. 3B), varying from 70% during the intensive phase to between 5 and 20% in the succeeding passages. The fluorescent appearance of positive cells was characteristically a slight granular and confluent staining of the cytoplasm (data not shown).

Measles virus-specific polypeptides and RNA in MV-308 cells. A parallel fluctuation with virus production was observed in the quantity of MV-specific proteins when specimens from all passages were analyzed by dot-blotting (Figs. 3C, 4B). Accordingly, the intensity of the individual polypeptides detected by the immunoblotting technique using rabbit anti-MV serum varied cyclically and no apparent differences between the polypeptide pattern of the MV-infected Vero cells and the

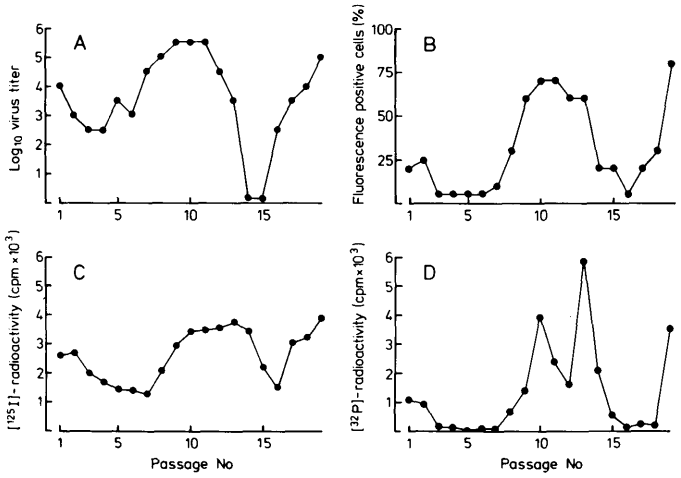

FIG. 3. Replication of measles virus in MV-308 hamster brain cells. The amount of extracellular virus (A), the proportional amount of antigen-positive cells (B), the quantity of measles virus-specific protein (C), and RNA (D) were determined at each of the 19 serial passages.

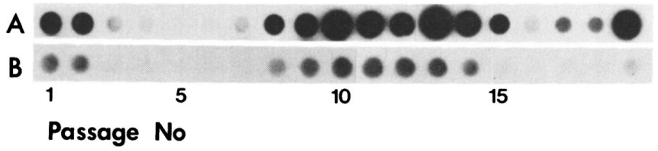

FIG. 4. Detection of measles virus RNA by spot hybridization (A) and measles virus proteins by dot-blotting techniques (B) in the 19 serial passages of MV-308 hamster brain cells.

polypeptide patterns of the serial MV 308 cell line passages were detected (Fig. 5).

The MV-308 hamster brain cell line and measles virus-infected Vero cells were metabolically radiolabeled with [^{35}S]methionine, and intracellular viral proteins were analyzed by SDS–polyacrylamide gel electrophoresis. Figure 6 shows a typical autoradiogram obtained. The proportional amount of the hemagglutinin glycoprotein H appears less when compared to its amount in MV-infected Vero cells.

The amount of MV RNA in the cells was determined by spot hybridization with a probe complementary to viral RNA. A clear fluctuation was detected also at the level of viral RNA (Figs. 3D, 4A) with a correlation to the fluctuation seen at the other levels of viral replication.

Discussion. MV has been a favorite model for the elucidation of the complex relationship between virus replication and host cell survival, i.e., persistence. An obvious reason for selecting MV as a model is the capability of the virus to induce persistent infections in clinical patients. Subacute sclerosing panencephalitis has been shown to be associated with paramyxovirus infections and the link between multiple sclerosis and these viruses still remains unclear.

During the last 10 years a variety of *in vitro* models of MV persistence have been established. One of the weak points in these models appears to be differing properties of each individual cell line. Additionally, the events in the host cell biology have not been characterized in detail. However, the crucial phenomenon in the maintenance of the persistent state must be a balance between cellular and viral activities. The cell model described in this report offers a constant fluctuating state between these phenomena.

The MV-308 persistently infected hamster brain cell line serves as a good *in vitro* model system to study the virus–host relationship in brain cells. The carrier state seems to be constant and the cell line has good growth properties, making possible the collection of specimens for parallel studies. The release of infectious virus fluctuates markedly in serial

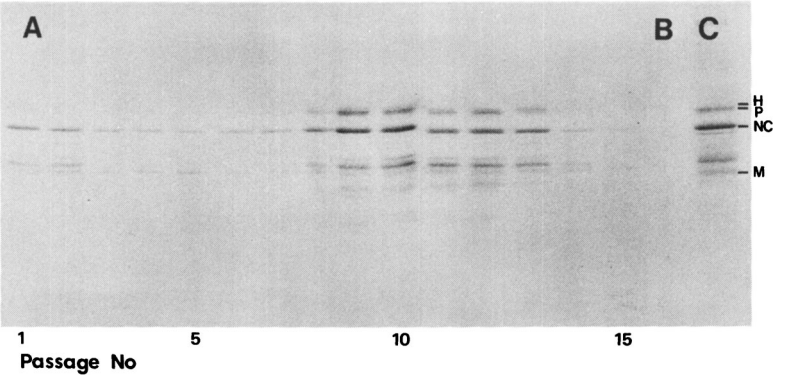

FIG. 5. Immunoblotting analysis of measles virus-specific polypeptides in the first 15 passages of MV-308 cells. Lane B is the control lane and lane C is measles virus-infected Vero cells. The polypeptides were analyzed by SDS–PAGE, transferred to a nitrocellulose filter, and probed with rabbit anti-measles serum and HRPO-conjugated anti-rabbit antibodies.

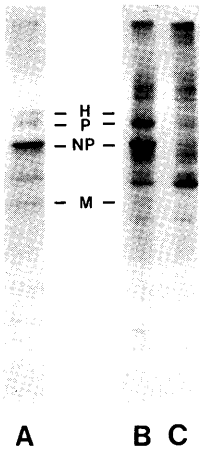

FIG. 6. Polypeptides synthesized in lytically infected Vero cells (A), in MV-308 hamster brain cells (B), and in uninfected control brain cells (C). The cells were pulse-labeled with [^{35}S]methionine for 30 min and polypeptides were analyzed by 10% SDS–PAGE. The major viral polypeptides are indicated.

passages, while the growth properties of the released virus are similar to the original wild-type virus. The reason for the fluctuation may be related to an event at the level of viral macromolecular synthesis or to the number of virus-producing cells. In order to examine the first possibility, we examined viral protein synthesis and the replication of viral RNA. The relative amount of virus-specific proteins in serial passages, as analyzed by dot-blot methods, fluctuated similarly with virus production. However, the immunoblot profile was almost identical to the polypeptide profile of the MV-infected Vero cells. On the other hand, the relative amount of the H glycoprotein synthesized in the persistently infected hamster brain cell line was lower than in the lytically infected Vero cells as determined by metabolic radiolabeling of the cells with [^{35}S]methionine. This result could not be confirmed by the immunoblotting technique as the immunological reactivity of the H glycoprotein on nitrocellulose filter (with the MV antiserum used) was found to be weak. Young *et al.* (15) have reported a similar decrease in synthesis of the H glycoprotein in a persistently infected HeLa cell line.

The replication of MV viral RNA was analyzed by a hybridization assay. The probe used detects both the genomic minus-strand RNA and the viral messenger RNA of positive polarity, as both plus and minus strands are found in virions. The assay showed that parallel fluctuation occurred also at the level of viral RNA synthesis. As the number of fluorescence-positive cells fluctuates, the alterations at the level of viral macromolecular synthesis may reflect the changes in the infection rate.

Our results favor the possibility that host cell-specific factor(s) may be responsible for the restriction of viral expression. Ehrnst (16) described the growth cycle dependent fluctuation of MV glycoproteins in nonneural cells. Miller and Carrigan (17) reported the reversal of inhibition of MV replication in neural cells by agents which affect cyclic nucleotide (cAMP and cCMP) metabolism. On the other hand, neural cells are known to contain abundant endogenous cAMP, the levels of which are closely correlated to the cell cycle. Our data do not allow us to make an unequivocal conclusion concerning the mechanism of MV persistence and fluctuation in hamster brain cells, but they clearly favor the involvement of a cellular mechanism.

We thank Ms. Marita Maaronen and Ms. Marjut Sarjovaara for excellent technical assistance and Ms. Taina Kivelä for secretarial help. We also are grateful to Drs. R. Marusyk and A. Salmi for critical comments during the preparation of the manuscript. This study was supported by the Sigrid Juselius Foundation.

1. Horta-Barbosa L, Fuccillo DA, Sever JL, Zeman W. Subacute sclerosing panencephalitis: Isolation of measles virus from a brain biopsy. Nature **221**:974, 1969.
2. Hall WW, Choppin PW. Evidence of lack of synthesis of the M polypeptide of measles virus in brain cells in subacute sclerosing panencephalitis. Virology **99**: 443–447, 1979.
3. Hall WW, Choppin PW. Measles-virus proteins in the brain tissue of patients with subacute sclerosing panencephalitis. N Engl J Med **304**:1152–1155, 1981.
4. Carter MJ, Willcocks MM, terMeulen V. Defective translation of measles virus matrix protein in a subacute sclerosing panencephalitis cell line. Nature (London) **305**:153–155, 1983.
5. Haase AT, Gantz D, Elbe B, Walker D, Stowring L, Ventura P, Blum H, Wiergrefe S, Zupancic M, Tourtellotte W, Gibbs CJ, Norrby E, Rozenblatt S. Natural history of restricted synthesis and expression of measles virus genes in subacute sclerosing panencephalitis. Proc Natl Acad Sci USA **82**:3020–3024, 1985.

6. Johnson KP, Norrby E, Swoveland P, Carrigan DR. Experimental subacute sclerosing panencephalitis: Selective disappearance of measles matrix protein from the central nervous system. J Infect Dis **144**: 161–169, 1981.
7. Payne FE, Baublis JV, Itabash HH. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. N Engl J Med **281**:585–589, 1969.
8. Breschkin AM, Morgan EM, McKimm J, Rapp F. SSPE-Biken: A naturally arising hemagglutination-defective mutant of measles virus. J Med Virol **4**:67–80, 1979.
9. Norrby E, Kristensson K, Brzosko WJ, Kapsenberg JG. Measles virus matrix protein detected by immune fluorescence with monoclonal antibodies in the brain of patients with subacute sclerosing panencephalitis. J Virol **56**:337–340, 1985.
10. Vainionpää R, Salmi A, Arstila P. Characterization of a hamster brain cell line persistently infected with measles virus. Arch Virol **71**:311–322, 1982.
11. Saraste J, Hedman K. Intracellular vesicles involved in the transport of Semliki Forest virus membrane proteins to the cell surface. EMBO J **2**:2001–2006, 1983.
12. Hyypiä T, Korkiamäki P, Vainionpää R. Replication of measles virus in human lymphocytes. J Exp Med **161**:1261–1271, 1985.
13. Maniatis T, Fritsch EF, Sambrook J. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.
14. Salonen R, Salmi A. An interferon assay based on yield reduction of vesicular stomatitis virus antigen. J Virol Methods **5**:143–149, 1983.
15. Young KKY, Heineke BE, Wechsler SL. M protein instability and lack of H protein processing associated with nonproductive persistent infection of HeLa cells by measles virus. Virology **143**:536–545, 1985.
16. Ehrnst A. Growth phase related loss of measles virus surface-associated antigens and cytotoxic susceptibility in persistently infected cell. J Gen Virol **45**:547–556, 1979.
17. Miller CA, Carrigan DR. Reversible repression and activation of measles virus infection in neural cells. Proc Natl Acad Sci USA **79**:1629–1633, 1982.

Received March 9, 1987. P.S.E.B.M. 1987, Vol. 186.
Accepted June 18, 1987.